DEVELOPMENT AND CHARACTERIZATION OF *Morinda citrifolia* PHYTOSOMAL GEL FOR TOPICAL APPLICATION

Dissertation submitted to
The Tamil Nadu Dr. M.G.R Medical University, Chennai
In partial fulfillment for the requirement of the degree of

MASTER OF PHARMACY

(Pharmaceutics)

APRIL- 2017

DEPARTMENT OF PHARMACEUTICS

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD, COIMBATORE- 641048

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Dr. A RAJASEKARAN, M.Pharm., Ph.D.,

PRINCIPAL,
KMCH COLLEGE OF PHARMACY,
KOVAI ESTATE, KALAPATTI ROAD,
COIMBATORE– 641048. (TN)

CERTIFICATE

This is to certify that this dissertation work entitled **"DEVELOPMENT AND CHARACTERIZATION OF *Morinda citrifolia* PHYTOSOMAL GEL FOR TOPICAL APPLICAION"** was carried out successfully by **Reg.no:261510901.** The work mentioned in the dissertation was carried out at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore- 641048, for the partial fulfillment for the Degree of Master of Pharmacy and is forward to The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Date:
Place: Coimbatore

Dr. A RAJASEKARAN, M.Pharm., Ph.D.,
Principal

GUIDE
Department of Pharmaceutics,
KMCH College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore- 641048.

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Date:
Place: Coimbatore

Dr. K.S.G. Arul Kumaran, M.Pharm., Ph.D.,
Department of Pharmaceutics

DECLARATION

I do hereby declare that this dissertation entitled **"DEVELOPMENT AND CHARACTERIZATION OF *Morinda citrifolia* PHYTOSOMAL GEL FOR TOPICAL APPLICAION"** submitted to the Tamil Nadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** was done at Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, during the year 2016-2017.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"DEVELOPMENT AND CHARACTERIZATION OF *Morinda citrifolia* PHYTOSOMAL GEL FOR TOPICAL APPLICAION"** submitted by **Reg.no:261510901** to the Tamil Nadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, and was evaluated by us during the academic year 2016– 2017.

Examination Centre: KMCH College of Pharmacy, Coimbatore – 48.

Date:

Internal Examiner **External Examiner**

Convener of Examination

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Reg.no:261510901

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ABBREVIATIONS

NDDS	Novel Drug Delivery Systems
%	Percentage
eq	Equation
$^{w}/_{w}$	Weight by weight
i.e.	That is
q.s.	Quantity Sufficient
et.al	et alii, 'and others'
Fig	Figure
e.g.	Example
mg	Milligram
µg	Microgram
nm	Nanometer
ml	Milliliters
cm	Centimeter
gm/cm^3	Grams per Cubic Centimeter
µg/ml	Microgram per milliliter
aq	Aqueous
PC	Phosphatidylcholine
Avg	Average
Hrs	Hours
pH	Hydrogen Ion Concentration
Abs	Absorbance
SEM	Scanning electron microscopy
CR	Cumulative Release
R^2	Regression Coefficient
Conc	Concentration
ºC	Degree centigrade
EE	Entrapment Efficieny
rpm	Rotations per minute

Min	Minute
Sec	Seconds
vs.	Versus
PPB	Plasma Protein Binding
BA	Bioavailability
n	Slope
V_d	Volume of Distribution
RH	Relative Humidity
WHO	World Health Organization
ICH	International Conference on Harmonisation

INTRODUCTION

Herbal medicine is one of the oldest and most universal system of health care system. The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care. Every nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies. [1] Modern herbal medicine is based upon the combination of traditional knowledge, clinical experience, understanding of medicinal science and scientific evidence of herbal medicine. People are slowly and gradually switching to alternative forms of medicine. One of these many alternative therapies include herbal system of medicine. It is made of from an extract taken from the plant parts (leaf, root, flower and bark). They are absolutely natural and safe form of curing illness form occurring repeatedly. They help in curing the ailment and are also known to prevent the illness from occurring repeatedly. Herbal medicines may have long curing periods, but they eradicate the illness from it and prevent any future episodes of the same.[2]

Despite criticism of herbal medicine among mainstream medical professionals , it is wise to remember that many common drugs we use today were derived from plant based sources .Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies , including opium , aspirin , digitalis and quinine . According to World Health Organization (WHO) approximately 25% of modern drugs used have been derived from plants. At least 7000 medical compounds in modern pharmacopoeia are derived from plants .Among the active compounds currently isolated from the higher plants and which are widely used in modern medicine today show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.[3]

Advantages of herbal system of medicines[4]:

- Lower risk of side effects
- Widespread availability
- Effectives with chronic medicine
- Low cost effectiveness make them all the more alluring

- Efficacious for life style diseases for prolonged period of time
- Natural detoxification process of the body is effectively enhanced by herbal medicine.
- These type of formulation are best for the people who are allergic to various types of drugs.
- These types of medicines do not have any types of side effects as they are free from chemicals.

Disadvantages of herbal system of medicines[5]:

- Bulk dosing.
- Poor stability in higher acidic pH, liver metabolism etc.
- Large molecular size limiting the absorption via passive diffusion.
- Poor lipid solubility hence preventing their ability to cross the lipid-rich biological membranes.
- High mount of raw material is required for processing the medicine.
- Isolation and purification of individual components from whole herbal extract lead to partial or total loss of therapeutic activity

These limitation lead to reduced bioavailability and hence, low therapeutic index of plant active constituents. Often, the natural synergy is gone which is due to chemically related constituents present in herbal extract. Hence considerable attention has been given to development of novel drug delivery system for herbal drugs.

Novel Herbal Drug Delivery System[6]

Novel herbal drug delivery system opens new way for delivery of herbal drugs at right place, at right concentration, for right period of time and also gives scientific evidence to verify the standardization of herbal drug. With the progress in all fields of science and technology, the dosage forms have evolved from simple pills to highly sophisticated technology intensive drug delivery systems, which are known as Novel Drug Delivery System (NDDS). In the past decades considerable attention has been focused on the development of novel drug delivery systems for herbal drugs. Herbal drugs are becoming more popular in the modern world for their ability to cure various diseases with less toxic effects and better therapeutic effects.

Importance of novel herbal drug delivery system[7]

Most of the active constituents present in the herbal drugs are flavonoids, glycosides etc. These are mainly hydrophilic molecules due to that they are limited in their effectiveness and are poorly absorbed when they are taken internally and when applied topically. Apart from that due to its larger molecular size which cannot be absorbed by passive diffusion and due to their poor lipid solubility limiting its ability to pass across the lipid-rich outer membranes of the enterocytes (the cells that line the small intestine) resulting poor bioavailability of drugs. Therefore, a larger dose is usually required for dosage regimens. These aspects constitute a drawback against the widespread usage of phyto medicines in the pharmaceutical field. The effectiveness of many herbal drugs is mainly based upon delivering an effective level of the active phyto constituents present in it. These can be overcome by suitable incorporation of the novel drug delivery technology to herbal extracts minimizes the drug degradation or pre systemic metabolism and serious side effects by accumulation of drugs to the non targeted areas and improves the ease of administration in patients.

Various phytochemical and phyto pharmacological studies proves that the compositions, therapeutics and overall health enchancing capacities of various plant extracts but there is a great interest and medical need for the improvement of bioavailability of large number of herbal drugs and plant extracts which is having poor lipid solubility and poor bioavailability[7].

Many herbal drugs unlike their extraordinary potential *in-vitro* finding shows less or no *in-vivo* actions as a reason of its poor lipid solubility and larger molecular size finally resulting poor absorption and bioavailability of the drug.[5] Numerous phytoconstituents present in it may produce a combined action of the phytoconstituents and various methods like purification and separation of the plant parts leads to a partial loss of specific activity due to the removal of chemically related substances contributing the activity of the main components present in it. Very often the chemical complexity of the extract is important for the bioavailability of the active components. Most of the plant constituents specifically phenolics are water soluble and so the major problem for less bioavailability is the inability to cross the lipid rich biological membranes.[8]

Novel drug delivery system is useful in delivering the herbal drug at a predetermined rate and delivery of drug at the site of action which reduces the side effects with increase in

bioavailability of drugs. In novel drug delivery technology control of the distribution of drugs is obtained by incorporating the drug in suitable carrier system or by converting the structure of the drug at molecular level. Incorporation of herbal drugs in the delivery system also aids to increase its solubility, enhanced stability, protection from toxicity, enhanced pharmacological activity, improved tissue macrophage distribution , sustained delivery and protection from physical and chemical degradation .For good bioavailability natural products must have a good balance between hydrophilicity and lipophilicity to cross lipid biological membranes.[5]

The novel carrier should ideally fulfill to requirements such as it should deliver the drug at the rate directed by the needs of the body and over the period of treatment secondly it should be a channel for the active entry of herbal drug to the site of action. Incorporating the herbal drugs into the novel drug delivery system reduces the repeated administration of drug to overcome non-compliance which helps to increase the therapeutic value by reducing toxicity and increases the bioavailability. Novel drug delivery admits to either prolonged drug action at a predetermined rate or by maintaining the relatively constant active drug level in the body with minimization of undesirable toxic effects.[1]

Advantages of Novel herbal drug delivery system[5]

- Help to increase the efficacy and reduce the side effect of various herbal compounds.
- Quantity of component becomes less with improving quality of drug effect.
- Fewer raw material are required to achieve the desire effect and control drug delivery to provide exact specification regarding drug dose form.
- Ready to use devices are acceptable in today's fast life style where time is important.
- Carry maximum amount of drug to the site of action by passing all barriers such as acidic pH of stomach
- Increases prolong circulation of drug into blood due to their small particle size.
- Reduce repeat dose administration.

Recent adavances in Novel Herbal Drug Delivery Systems[5-11]

Various approaches in case of novel herbal drug delivery includes different types of formulations such as liposomes , phytosomes , niosomes , nanoparticles , microspheres ,

transferosomes , ethosomes , herbal transdermal patches and proniosome etc. are discussed as follows :

(A) Liposomes

The liposomes are spherical particles that encapsulate a fraction of the solvent, in which they freely diffuse (float) into their interior. They can have one, several or multiple concentric membranes. Liposomes are constructed of polar lipids which are characterized by having a lipophilic and hydrophilic group on the same molecule. Upon interaction with water, polar lipids self assemble and form self organized colloidal particles .Liposome depict the hydrophilic heads of the amphiphile orienting towards the water compartment while the lipophilic tails orient away from the water towards the center of the vesicle, thus forming a bilayer . Consequently water soluble compounds are entrapped in the water compartment and lipid soluble compounds aggregate in the lipid section .Thus liposomes can encapsulate both hydrophilic and lipophilic materials. They are usually formed from phospholipids, have been used to change the pharmacokinetic profile of not only drugs but herbs vitamins and enzymes.

(B) Niosomes

Niosomes are microscopic lamellar structures formed on admixture of a nonionic surfactant, cholesterol and a charge inducing agent with subsequent hydration in aqueous media. Niosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility‟s. Niosomes have been evaluated in many pharmaceutical applications. In such therapeutic applications, important advantages of using niosomes include their ability to reduce systemic toxicity by encapsulation of treatment agents and minimize clearance of such agents from the body by slow drug release**.**

(C) Nanoparticles

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The major goals in designing nano particles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Nanoparticles

offer some specific advantages such as they help to increase the stability of drugs/proteins and possess useful controlled release properties. It can be modified to achieve both active and passive targeting; drug loading is very high and can be administered by various routes such as parenteral, nasal, intra ocular and oral routes.

(D) Microspheres

Microspheres are solid, almost spherical in nature, having a diameter in the range of 1 um to 1000um. Microspheres are having wide commercial applications including sustained drug delivery, overcome handling issues with potent molecules and improved targeting at the active site in desired concentration to maintain overall effective plasma concentration for a longer period of time. Microspheres are widely used as drug delivery carrier for targeting of various agents that cause dose-dependent adverse effects. In recent times, microspheres are widely used to enhance the therapeutic potential of various poorly soluble phytoconstituets.

(E) Transferosomes

Transfersome carrier is an artificial vesicle which resembles the natural cell vesicle. Thus it is suitable for targeted and controlled drug delivery. Transfersome is a highly adaptable and stress-responsive, complex aggregate. It is an ultra-deformable vesicle which possesses an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimising. This enables the Transfersome to cross various transport barriers efficiently, and then act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. These self-optimized aggregates, with the ultra-flexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency.

(F)Ethosomes

Ethosomes are developed by mixture of phospholipids and high concentration of ethanol. This carrier can penetrate through the skin deeply lead to improve drug delivery into deeper layer of skin and in blood circulation. They may form multilamellar vesicles and have a high entrapment capacity for molecules of various lipophilicities. They are mainly used as drug carriers for a

range of small molecules,various phytoconstituents , peptides, proteins and vaccines . They show increase in their permeability through the skin by fluidizing the lipid domain of the skin.

(F)Herbal transdermal patches

Transdermal drug delivery systems facilitate the passage of therapeutic quantities of drug substances through the skin and into the general circulation for their systemic effects. Herbal transdermal patches are medicated adhesive pad designed to release active ingredients at a constant rate over a period of several hours or days after application to skin. Herbal penetration enhancers like some terpenes are found to be potential enough to replace the conventionally available penetration enhancers.

(G) Phytosomes [12,16]

The phytosome are newly introduced structures, which contain the active ingredients of herb surrounded and bound by phospholipids. These are the newly introduced patented technology developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes. It results from reaction of stoichiometric amount of phospholipid mostly phosphatidylcholine with a standardized herbal extract in an aprotic solvent. It mainly contains the bioactive phytoconstituents of herb surrounds and bound by a lipid.

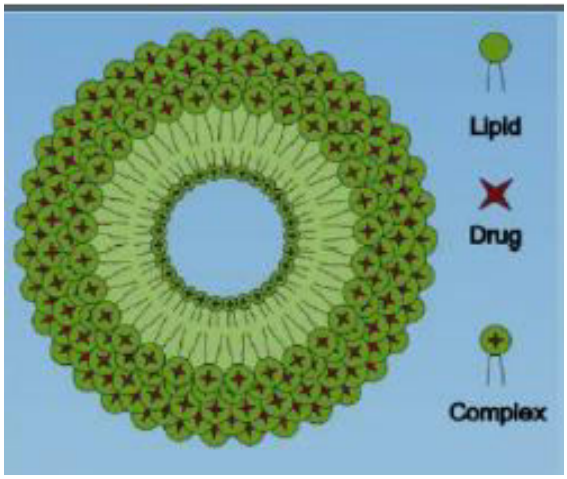

Figure no : 1 The phytosome complex

Most of phytoconstituents of herbal extracts are water soluble and poorly miscible with oils and other lipids. Lipid solubility and molecular size of phytoconstituents are the major limiting factors for molecule to pass the biological membrane to be absorbed systemically following the

oral or topical administration. The bioavailability of phytoconstituents can be increased by use of novel drug delivery system, which can increase the phytoconstituents solubility in gastrointestinal fluid as well as capacity to cross lipid rich biological membrane.[6] Phytosome of herbal extract is generally more bioavailable than a simple herbal extract due to its enhanced capacity to cross the lipid-rich biomembranes. Phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, and without compromising nutrient safety. They have improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute diseases as well as in pharmaceutical and cosmetic compositions.[13]

Phosphatidylcholine: as carrier for herbal drugs[20,22]

Phosphatidylcholine is the principal molecular building block of cell membranes miscible both in water and in oil environments, and is well absorbed when taken by orally or topically . They are a major component of biological membrane and can be isolated from either egg yolk or soy beans from which it is mechanically or chemically extracted using hexane. The choline head of the phosphatidylcholine molecule binds to these compounds while the fat-soluble phosphatidyl portion comprising the body and tail then envelopes the choline-bound material. As a result a little microsphere or cell like structure will appear.

The phytosome technology creates intermolecular bonding between individual polyphenol molecules and one or more molecules of the phosphatidylcholine or phospholipids. A bond is formed between these two molecules, creating a hybrid molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocyte's outer cell membrane so they are more bioavailable as compared with conventional herbal extracts owing to their enhanced capacity to cross the lipid-rich biomembranes and, finally, reach the blood.[15]

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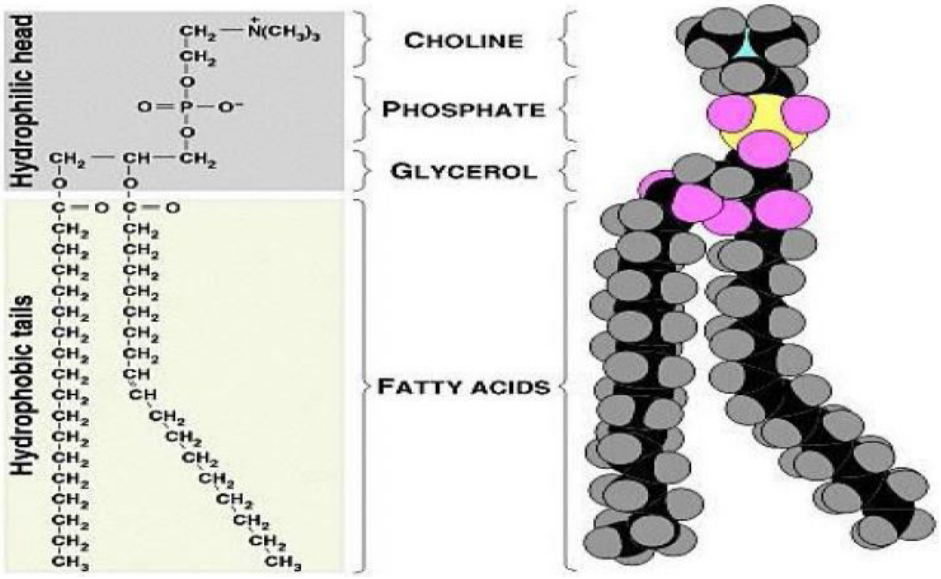


Figure no: 2 Structure of phosphatidylcholine

Advantages Of Phytosomes [12,16]

1. Potential enhancement of bioavailability.
2. Herbal phytosome process produces a little cell whereby the valuable components of the herbal extracts are protected from destruction by digestive secretions and gut bacteria.
3. Pharmacologically Assured delivery to the different biological tissues.
4. No compromise of nutrient safety.
5. Less dose requirement is due to absorption of chief constituent.
6. Drug loading efficiency is so high and more over predetermined because drug itself in conjugation with lipids is forming vesicles.
7. No problem of drug entrapment.
8. Phytosomes shows better stability profile because chemical bonds are formed between phosphatidylcholine molecules and phytoconstituents.
9. Phosphatidylcholine used in the phytosome process which acting as a carrier and also nourishes the skin, because it is essential part of cell membrane.
10. Phytosome is also superior to liposomes in skin care products.
11. Significantly gives greater clinical benefit than liposomes.
12. The structure of phytosome elicits peculiar properties and advantages in cosmetic application.

13. Significantly enhanced ability of phyosome to cross cell membranes and enter cells.
14. Their low solubility in aqueous media allows the formation of stable emulsions or creams
15. Relatively simple to manufacture with no complicated technical investment required for the production of phytosomes.

Properties of phytosomes [16,17,19]

1. Chemical properties

Phytosomal complex is obtained by reaction of stoichiometric amounts of phospholipid and the substrate in an appropriate solvent. On the basis of spectroscopic data it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functionalities of the substrate. in phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane This can be deduced from the comparison of the NMR of the complex with those of the pure precursors. The signals of the fatty chain are almost unchanged. Such evidences inferred that the two long aliphatics chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and the phytoconstituent.

2. Biological Properties

Phytosome are advanced forms of herbal products that are better absorbed, utilized and as a result produce better results than conventional herbal extracts the increased bioavailability of the phytosome over the non complexed botanical derivatives has been demonstrated by pharmacokinetics studies or by pharmacodynamic tests in experimental animals and in human subjects.

3. Pharmacological properties

Phytosomes are advanced form of herbal products that are better absoebed, utilised and as a result produce better results than conventional herbal extracts. The increased bioavailability of phytosomes over noncomplexed botanical derivatives has been demonstrated by pharmacokinetic studies or by pharmacodyanamic tests in experimental amials and human subjects.

PHYTOSOMES: A Novel approach towards topical drug delivery system [18]

The skin is the biggest organ of the human body and provides a protective barrier against various harmful microbes, chemicals and ultraviolet radiation. Natural plant products have been formulated to heal and prevent dry skin, treat skin conditions such as eczema and acne and retard the aging process. As we grow older, the process of cell replication and many other complex cellular pathways breaks down and the body begins to make mistakes. In the same way that our reactions slow down as we age, so too our cells make errors and function more slowly. Young vibrant elastic collagen becomes progressively sluggish and inelastic, skin becomes more lined and wrinkled, colour and freshness are slowly replaced with dull, lifeless skin often speckled with discoloured patches where the deposition of melanin has become uneven and erratic.

Plant flavonoids have local action on some diseases like inflammation, oedema, pain, fungal infections etc. But their use in topical application is restricted due to poor absorption through skin. Phytosomes improve absorption of phytoconstituents through skin, to regulate the physiology of skin compositions. The improvement in the functioning of skin suggests the functional importance of the phytosomes.[8] Generally the passage of the compounds anchored to phospholipids takes place through interaction with cutaneous structure, which influences the release of the phytoconstituents. The rate of absorption from the complex is dramatically enhanced without damaging the epidermis, which suggesting potential use of phytoconstituent-phospholipids complex for cosmetic value in skin as well as for systemic function via skin. Therefore application of natural molecules in form of phytosome improves its absorption, nourishes the skin and act as a topical drug delivery system.

Skin Structure and Skin Alterations Due to Aging[18]

Structure of skin is shown figure no: 3. The aging of human skin is primarily caused by exposure to the ultraviolet radiation of the sun. There are two main process of skin aging: intrinsic and extrinsic. Intrinsic aging reflects the genetic background of an individual and results from the passes of time.

It is inevitable; thus, it is beyond voluntary control. Extrinsic aging is caused by external factors such as smoking, excessive use of alcohol, poor nutrition and sun exposure.

This process then is not inevitable and referred as pre mature skin aging. Such types of aging are successfully protected by use of functional cosmetics.

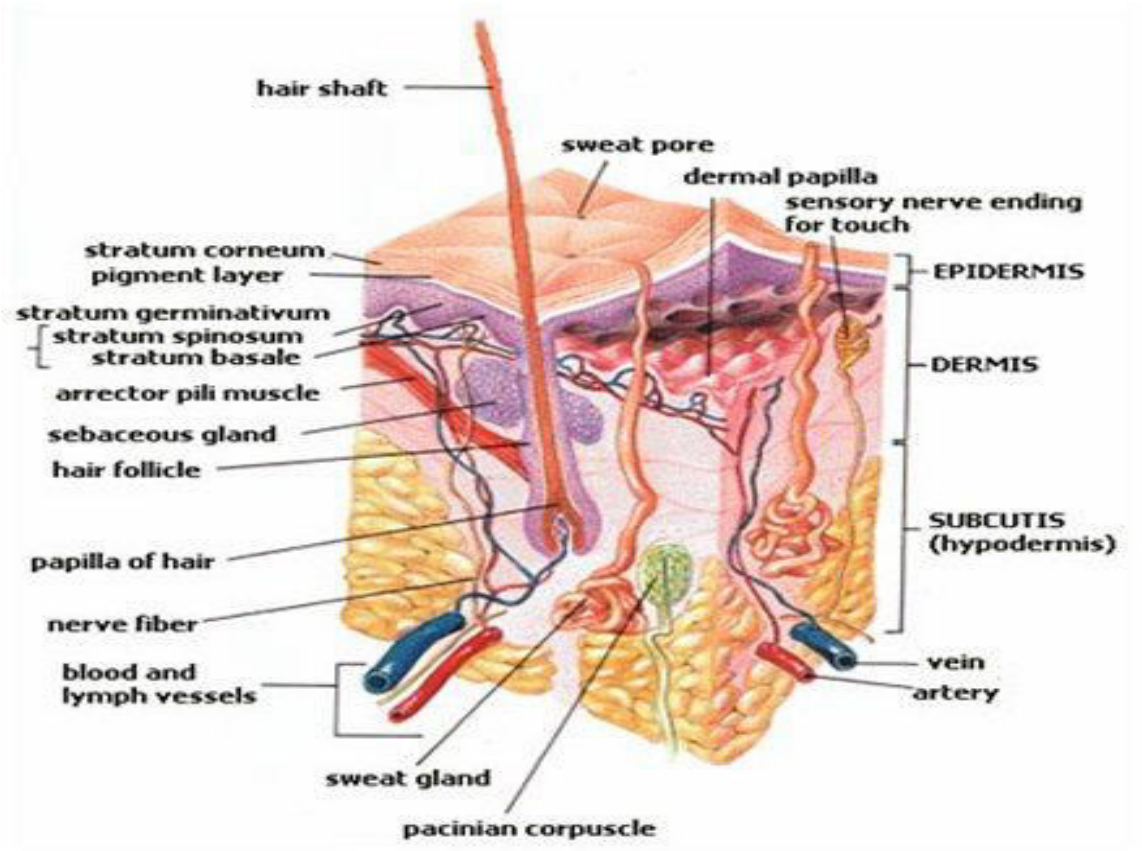


Figure no: 3 Structure Of Skin

Signs of Intrinsic Aging

- Fine wrinkles.
- Thin and transparent skin
- Loss of underlying fat.
- Bones shrink away from the skin due to bone loss, which causes sagging skin.
- Dry skin that may itch.
- Inability to sweat sufficiently to cool the skin.

• Graying hair that eventually turns white.

• Hair loss.

• Unwanted hair.

•Nail plate thins, the half moons disappear and ridges develops.

Signs of Extrinsic Aging

•Wrinkles

•Toxicity.

•Uneven pigmentation

•Brown spots and leathery appearance.

•Chronologically aged skin.

Other Skin Complications

• Eczema, also known as atopic dermatitis, is a skin disorder characterized by redness, swelling, itching and scaling.

•Acne is an inflammatory disease of the sebaceous glands and hair follicles of the skin that causes pimples or pustules, especially on the face.

Selection of PhytoconstituentsFormulationConsiderations[21]

Following are selection criteria's of phytoconstituent from herbal extract for phytosomes preparation and formulation considerations for phytosome development.

(A) Selection of herbal extracts

Herbal extracts posses various properties such as photo-protection, hepato-protection, anti-aging, moisturizing, and antioxidant, astringent, anti-irritant, and antimicrobial. Because of such properties they produces healing, softening, rejuvenating, and sunscreen effect on skin and improve pharmacological and pharmacokinetic profile in the body. After detailed literature survey of herbs and correlation of activity of herbal compounds based on chemical classes such as flavonoids, monoterpenes, polyphenols, indols and organosulfides, one can select herbal extracts on the basis of their nature, availability, estimation method, stability and utility of developed formulation as well as reported previous research.

(B)Nature of phytoconstituents

Solubility is important criterion for the development of novel formulations. According to the nature of the phytoconstituents, that is hydrophilic or lipophilic, best suitable formulation can be selected.

Preparation of phytosomes [22,23]

1 . Solvent Evaporation Method:

The particular quantity of drug and phospholipids can be taken in a spherical bottom flask and reflux with specific solvent at a temperature of 50 to 60˚c for 2 hour . The mixture may be concentrated to 5 to 10 ml in order to get a precipitate which can be filtered and collected. The dried precipitate phytosome loaded can be placed in amber colored glass bottle and stored under room temperature.

2. Rotary Evaporation Technique:

The particular quantity of drug and phospholipids can be dissolved in specific solvent in a rotary spherical bottom flask followed by stirring for 3 hours at a temperature not exceeding 40˚c . Thin film of the sample can be obtained to which a solvent is added and continuously stirred using a magnetic stirrer. The precipitate phytosome loaded obtained can be collected placed in amber colored glass bottle and stored under room temperature .**3. Antisolvent Precipitation Technique:**

The particular quantity of drug , phospholipids taken into a spherical bottom flask and reflux with specific solvent at a temperature not exceeding 60˚c for 2 hour . The mixture can concentrated to 5 to 10 ml . Solvent can carefully added with continuous stirring to get precipitate which has filtered and collected , stored in vacuum desiccators overnight . The dried precipitate is crushed in mortar and sieved through #100 meshes . The dried precipitate phytosome loaded can be placed in amber colored glass bottle and stored at room temperature.

Characterization of phytosomes[22,15] :-

1. Determination of % yield: Determination of % yield of phytosome complex was calculated by the following formula:

(%) Yield = (Practical yield) × 100 (Theoretical yield)

2. Determination of drug content: Drug content of phytosome complex was determined by dissolving accurately weighed quantity of complex in a solvent and after suitable dilution absorbance was determined by UV –Spectrophotometer and drug content was determined.

3. Entrapment efficiency: The entrapment efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultracentrifugation technique.

4. Solubility Studies: Solubility of the drug, phospholipids, their physical mixture and the pharmacosomes can be evaluated. The apparent partition coefficient can be determined by the shake-flask method where two phases are mutually saturated before use. Equal volumes of buffer solutions with a different pH (from 2.0 to 7.4) and 1-octanol containing phospholipid complex are mixed properly in the screw capped penicillin bottles and equilibrated under constant shaking at 37°C for 24hours. After separating the aqueous phase, the concentration of drug in this aqueous phase is determined by HPLC or UV spectrophotometry.

5. Scanning electron microscopy (SEM): Scanning electron microscopy has been used to determine particle size distribution and surface morphology of the complexes.

6. *In-vivo* and *In-vitro* Evaluations

Depending upon the expected therapeutic activity of biologically active constituents, models of *in-vivo* and *in-vitro* evaluations are carried out. In-vitro dissolution studies can be done with media of different pH in a standard dissolution apparatus to determine the pH dependent dissolution profile.

***Morinda citrifolia*[26,27]**

Morinda citrifolia L. (Rubiaceae), commonly known as Noni, has been extensively used in folk medicine by the Polynesians for over 2000 years. It is having a broad range of therapeutic effects like antiviral, antibacterial, antifungal, antitumor, anthelmintics, analgesic, hypotensive, wound healing, anti-inflammatory, anti cancer anti oxidant, immune enhancing effects.

Phytochemical investigations have shown that Noni contains a number of phenolic compounds, in particular coumarins and flavonoids, and iridoids which are reported to have anti-oxidant, anti-inflammatory and neuroprotective effects. But extraction of individual compound from the extract often exhibits limited clinical utility as the synergistic effect of various natural

ingredients gets lost. They generally constitute polyphenols and flavonoids which show poor absorption due to their multiple ring structures unsuitable for passive diffusion or lack of carrier mediated transport or poor miscibility in water/lipids.

Phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, and without compromising nutrient safety. The water-soluble phytomolecules (mainly flavonoids and other polyphenols) can be converted into lipid-friendly complexes, by reacting herbal drugs with phospholipid. They are more bioavailable as compared with simple herbal extracts owing to their enhanced capacity to cross the lipid-rich biomembranes and, finally, reach in the blood circulation. The phospholipid mainly used to make phytosomes, is phosphatidylcholine . Phytosomes are prepared from the reaction of a stoichiometric amount of the phospholipid (phosphatidylcholine) with the standardized extract or polyphenolic constituents.[12]

Based on the above observations, it was concluded to formulate phytosomes of Morinda citrifolia extract and evaluated for the improvement in drug loading and drug release. Findings of the research are included in this thesis.

REVIEW OF LITERATURE

Literature Review About Phytosome

K. Rajashekar *et al* (2015) .,[41] Prepared and evaluated topical phytosomal gel of *Woodfordia fruticosa* extract (WFE). Dried flowers of the plant were subjected to soxhlation using 80% methanol. The extract was complexed with soyalecithin in various drug:lipid ratios of 1:1, 1:2, 1:3, 1:4 and 1:5. Phytosomal complex of WFE was prepared by Ethanol method and Reflux method. All formulations were evaluated for vesicle formation, % yield, Entrapment efficiency and *in-vitro* drug release studies. Drug : lipid ratio of 1:3 (E3) was suitable as it resulted in greater entrapment efficiency and *in-vitro* drug release Characterization of phytosomal complex (E3) revealed vesicle size and Zeta potential to be 213.1nm and 31.5mv indicating stability of the complex. Phytosomal gels of WPC E3 were formulated using different polymers (Carbopol 934, HPMC K4M, HPMC K100) in various concentrations. All gels were evaluated for homogeneity, pH, viscosity, drug content and *in-vitro* drug release studies. In vitro antioxidant activity of extract, phytosome and gel are in following order E3>PCG1>WFE. The data obtained in this study revealed that the objective has been met. Results of comparative study indicated that better solubility, increased antioxidant activity& in vitro drug release of phytosomal gel over crude extract.

Keerthi B *et al* (2014).,[42] Formulated and evaluated capsules of Ashwagandha phytosomes. Two methods have been attempted in preparing Ashwagandha phytosomes complex ethanol method and reflux method. Ashwagandha phytosomes complex in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) were prepared using ethanol as a reaction medium. Ashwagandha phytosome complexes were characterized by particle size, zeta potential, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and *in vitro* drug release. The results showed that the average particle size and zeta potential of optimized Ashwagandha phytosomes formulation were 98.4nm and −28.7 mV. *In vitro* drug release studies revealed that the cumulative % drug release of capsules of Ashwagandha phytosomes was found to be 76.8%. Antioxidant activity of Ashwagandha phytosomes was evaluated by reducing power method. The results showed that the Ashwagandha phytosome complex exhibited more antioxidant activity compared to the Ashwagandha extract. Hence it was concluded that Ashwagandha phytosomes serve as useful novel drug delivery system and provide more bioavailability than conventional formulations.

Rudra Pratap Singh *et al* (2015).,[43] Prepared and evaluated topical phytosomal gel of lawsone. Different phytosome complexes of lawsone containing molar ratio of 1:1, 1:2, 2:1 and 2:2 of lawsone and soya lecithin were prepared by the antisolvent precipitation technique. The phytosome was characterized by SEM, DSC and FTIR. Antifungal activity of phytosome of lawsone was evaluated on *Candida albicans* fungi by using ketoconazole as standard drug. The *in-vitro* permeation study was done on rat skin. The anti-inflammatory activity was evaluated in male wistar rats. SEM and DSC data showed that phytosome complex of lawsone has irregular size vesicles consisting of soya lecithin and lawsone was found to be intercalated in the lipid layer. Antifungal activity of phytosome complex (1:1) showed the biggest zone of inhibition as compared to phytosome complex (1:2), plant drug and standard drug ketoconazole after 3 days. *Ex-vivo* permeation study of phytosome gel of lawsone through excised rat skin showed cumulative drug permeation up to 6 h.

Asit R Sahu *et al* (2015).,[44] Formulated boswellic acid loaded phytosome . Phytosomal formulations were developed using different concentration of Cholesterol (1-3%) and ethanol (20-40%) then optimized and characterized. Particle size, entrapment efficiency and vesicular shape were determined by Malvern Zetasizer, Ultracentrifugation and Scanning Electron Microscopy, respectively. Particle size varied from 179 to 514.8 nm depending on the concentrations of Cholesterol and ethanol. Entrapment efficiencies were exhibited of 39.8 ± 3.7% to 74.2 ± 4.3 %, where it increased with concentration of cholesterol and ethanol increased. Photomicrographs revealed that optimized Phytosomes were spherical in shape and uniform in size. Based on minimum particle size and maximum entrapment efficiency E6 (2% of Cholesterol concentration and 40% of ethanol concentration) was selected as optimization Phytosomal formulation.

Ashwini S Dhase *et al* (2015).,[45] Phytosomes was successfully prepared by solvent extraction method. Firstly leaves of *A.marmelos* were extracted with petroleum ether and methanol by soxhlet extraction. Then phytosomes batches were prepared by solvent evaporation method. F3 formulation selected as optimized formulation and futher evaluated it for particle size digital microscopy, SEM, TEM, FTIR, DSC, XRD analysis. Comparative evaluation ofantioxidant, antiproliferative and anticancer activity of extract and phytosome was carriedout. From above studies we are concluded that phytosomes has better physical characteristics ascompared to that

of methanolic extract of leaves of *A.marmelos*. Phytosomes has nearly same antioxidant, antiproliferative and anticancer activity as that of methanolic extract of leaves of *A.marmelos.*

Asija Sangeeta *et al* (2012).,[46] *Prosopis cineraria* extract in combination with the phospholipids was developed to overcome the limitation of absorption and bioavailability and to improve the lipophilic properties of *Prosopis cineraria* extract. Phytosomes were prepared by rotary evaporator method. The physicochemical properties of the phytosomes including infrared spectrophotometry, % yield, solubility and apparent partition coefficient were determined. In addition, drug content and *in vitro* dissolution was evaluated. The results of infrared spectra showed that there was some interaction between drug (*Prosopis cineraria* methanolic exract) and phospholipid (soya lecithin) in the complex (phytosomes), but no new characteristic absorption peaks were observed, indicating that no new covalent bonds were formed.

A. Sumathi *et al* (2015) .,[47] Designed and developed phytosomes of *Nymphaea nouchal* (Nn*) and Trichosanthes dioica* (Td), by reacting phosphatidyl ethanolamine (PE) in tetrahydrofuran with the selected botanical derivatives in dioxane:methanol (7:3) solvent system. Different Nn/Td:PE molar ratios 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10 were employed using solvent evaporation technique. *In vitro* appraisal encompassed differential calorimetry, infra red spectroscopy, particle size, drug content, diffusion and stability studies. The results revealed that the optimized phytosomal carriers, PE(Nn/Td) exhibited the mean particle size of 268 nm and good *in vitro* stability in the ratio of 1:8. It also exhibited significant enhancement in diffusion rate compared to crude drug mixture and standard (Levimasole). Thus, the phytosomal carrier (PE) with 89 % of entrapped drug could be successfully tailored for Nn/Td with improved *in vitro* release characteristics which is promising for increasing drug delivery and decreasing the effect of exogenous factors.

Malay K Das *et al* (2014).,[48] Rutin phytosomes (RN-P) were developed and characterized to establish its feasibility for transdermal application in inflammatory conditions. Phytosomes were prepared in five molar ratios of Rutin (0.5 - 1.0) to Phosphatidylcholine (1.0 - 0.5). All RN-Ps showed aqueous solubility higher than pure Rutin. Partition coefficient results indicated the lipophilic nature of free Rutin as well as all RN-Ps with most satisfactory value found at 3.11 ± 0.08 with F3 formulation. Discrete vesicular structures of RN-Ps observed in TEM study. Results of the FT-IR, DSC and XRD studies confirmed the phyto-phospholipid complex formation. XRD reports revealed the reduction in crystallinity of Rutin when in phytosomes form with F3 found

to be the least crystalline. SEM studies confirmed the disappearance of rod shaped crystals of Rutin in phytosome formulations. The *ex-vivo* skin permeation study across excised rat abdominal skin confirmed the higher permeability of RN-Ps (33 ± 1.33 %) over pure Rutin (13± 0.87 %). The observations made in the present work suggest that phyto-phospholipid complex of Rutin can increase its skin uptake to treat inflammatory conditions in arthritis, rheumatism, athletic aches and may able to deliver the drug for a long duration avoiding the problems associated with oral administration.

Sandeep Arora *et al* (2013).,[49] Formulated and characterized phytosomal complex tablets for sustained delivery of *Phyllanthus amarus* complex. Phyllanthin, one of the active lignin present in this plant species was isolated from the aerial parts, by silica gel column chromatography employing gradient elution with hexane–ethyl acetate solvent mixture. It was obtained in high yields (1.23%), compared to reported procedures and the purity was ascertained by HPTLC analysis. Phyllanthin was characterized for M.P, UV–Visible spectrophotometry, FT-IR, 1H NMR, 13C and NMR analysis. Release kinetics was evaluated by using United States Pharmacopeia (USP)-22 type I dissolution apparatus. Scanning electron microscopy was used to visualize the effect of dissolution medium on matrix tablet surface. HPTLC was carried out for quantitative and qualitative estimation of Phyllanthin in *Phyllanthus amarus* and Rf of phyllanthin was found to be about 0.25. The content was found to be maximum for phytosomal complex of phyllanthus formed by vaccum drying of 1:1 drug excipient ratio. The in-vitro drug release study revealed that optimized formulation sustained the drug release for 12 hr (88.1% ± 4.1% release). Fitting the in vitro drug release data to Korsmeyer equation indicated that diffusion along with erosion could be the mechanism of drug release.

Diogo Matias *et al* (2015).,[50] Formulated and characterized phytosomes containing a bioactive extract from *Plectranthus madagascariensis* and optimization of the preparation method. Different formulations and process parameters were studied. It was observed that smaller and more uniform particles were obtained using acetone as solvent, a reaction time of two hours, and the addition of 2.5% molar concentration of cholesterol. The optimally prepared phytosomes had a diameter of 191.3 ± 75.3 nm with a polydispersity index of 0.243 ± 0.18, and a spherical shape with amorphous appearance. These nanosystems were able to encapsulate 92.8% of the extract, as evaluated by HPLC, relative to 7α,6β-dihydroxyroyleanona, the main extract component. This

study suggests a future application of those phytosomes in the delivery of bioactive agents with therapeutic interest.

Parul A. Ittadwar *et al* (2016).,[51] Umbelliferone was successfully complexed with phosphatidylcholine to form phytosome. The preformulation studies confirmed identification of umbelliferone. The complex was successfully formulated by solvent evaporation method using Box-Behnken experimental design and batch was optimised. The optimised batch was evaluated for % practical yield, complexation rate, drug content and the results were within the range. The complex showed better solubility than the drug in both phases. The complex was found to show higher *in vitro* antioxidant activity than the drug at the same concentrations. The HPTLC, DSC, FT-IR, SEM, XRD and NMR study confirmed the successful formation of the complex. The *ex vivo* and *in vitro* permeation studies showed better release for phytosomal complex than the drug. The animal study was carried out for the photoprotective effect of complex and the effect was evaluated by estimating the antioxidant enzymes. The phytosomal complex was better able to protect the skin and then antioxidant enzymes than the drug. The stability study revealed that there were no significant changes in the formulation over the period of three months. Hence this study suggests that umbelliferone in novel drug delivery system i.e. phytosomal form, produces a better therapeutic effect than the drug alone.

Ahmed N.Allam *et al* (2015).,[52] Curcumin phytosomes were prepared by a simple solvent evaporation method where free flowing powder was obtained in addition to a newly developed semisolid formulation to increase curcumin content in soft gels. Phytosomal powder was characterized in terms of drug content and zeta potential. Thirteen different soft gel formulations were developed using oils such as Miglyol 812, castor oil and oleic acid, a hydrophilic vehicle such as PEG 400 and bioactive surfactants such as Cremophor EL and KLS P 124. Selected formulations were characterized in terms of curcumin *in vitro* dissolution. TEM analysis revealed good stability and a spherical, self-closed structure of curcumin phytosomes in complex formulations. Stability studies of chosen formulations prepared using the hydrophilic vehicle revealed a stable curcumin dissolution pattern. In contrast, a dramatic decrease in curcumin dissolution was observed in case of phytosomes formulated in oily vehicles.

Jadhav S.J. *et al* (2016).,[53] Formulated and characterized phytosomes of *Butea monosperma* phytosome by solvent evaporation technique . Different phytosomal ration of 1:0 , 1:0.8 , 1:1 , 1:2 were prepared and 1:2 ratio was optimized for the further studies .Phytosome complexes

were characterized by particle size, zeta potential, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and *in vitro* drug release and free radical scavenging activity by DPPH model . In-vitro drug release study of phytosome extended release pattern show 6 hr of 80.36 release . The synergistic effect was determined by free radical scavenging activity of BM phytosome using DPPH model . It was concluded that phytosome were successfully prepared and encapsulated shows extended release pattern with enchanced free radical scavenging activity.

Alisha Pereira *et al* (2015).,[54] Developed a phospholipid complex of amla extract that enhances the delivery of polyphenols into the skin. The amla extract phytosomes prepared, had an entrapment efficiency of 94.03±0.10 %. The characterization studies showed that constituents of amla extract successfully formed a complex with phospholipids. The complex was incorporated into a cream formulation, which was found to be stable. The *ex-vivo* diffusion studies of cream showed that administration of the amla extract via phospholipid complexes gave better skin retention compared to the conventional cream. Thus, phospholipid complex of amla extract gave a prolonged antioxidant effect compared to the conventional cream.

Prasuna Sundari Pingali *et al* (2015).,[55] Formulated and evaluated miconazole loaded topical phytosomal gels. This study was mainly based on the use of phytosome as carrier for drug loading of Miconazole(MCZ). Schrebera root bark extract (SRE) was used to formulate miconazole loaded phytosomal complexes (MPC's). *Schrebera swietenioides* (Oleaceae) root bark methanolic extract (SRE) was prepared by soxhlation. Miconazole nitrate (MCZ) and SRE were complexed using soyalecithin in definite drug:lipid ratios by ethanol method. All Miconazole loaded phytosomal complexes were evaluated and characterized for entrapment efficiency, in vitro release, vesicle size, vesicle stability and SEM. Blank phytosomes (SRE-soyalecithin complex) and drug-loaded phytosomes (MCZ loaded SRE phytosomes) were included for comparison. Further, the optimised MPC's were formulated as gels using varying polymers in differing concentrations and were assessed for their homogeneity, pH, drug content and in vitro permeation using Franz diffusion cell. The optimized formulation F4 was subjected to stability studies as per ICH guidelines. Microscopical examination of MPC's revealed spherical shape and uniform size. MP1B and MP6B were optimized based on their in vitro drug release profile. The evaluation of prepared phytosomal gels of MP1B and MP6B indicated F4 to be better with 93.3 % drug content and 92.54% cumulative drug release of MCZ in 12hrs.

Miconazole was successfully loaded into the SRE-phytosomal complex. The results demonstrated that other poorly soluble drugs can be further be explored for better therapeutic benefit.

Prasanna Habbu *et al* (2015).,[56] A phytoformulation, *Allium cepa*-phospholipid complex (ACP) was prepared and characterized by SEM, DSC and FT-IR. It was then screened for *in vitro* free radical scavenging, antidiabetic activity (50 mg/kg and 100 mg/kg, p.o.) in normoglycemic and STZ induced diabetic animals. Concentration of quercetin in ACP was estimated in serum by HPLC. SEM data showed that ACP has irregular size vesicles consisting of HSPC and ACE intercalated in the lipid layer. ACP showed one endothermal peak in DSC studies. ACP showed good *in vitro* antioxidant activity. In OGTT, treatment with ACE (*Allium cepa* extract), ACP and glibenclamide significantly improved the glucose tolerance in normal animals. In STZ induced diabetic rats, a single dose of ACE and ACP treatment exhibited reduction in SG levels at different time intervals compared to basal levels. Administration of both the doses of ACE and ACP for fifteen days exhibited greater percentage reduction in glycemia and restored to near normal value of all tested lipid parameters. Higher serum concentration of quercetin was observed for ACP in bioavailability studies as compared to ACE. The studies revealed that ACP has shown significant antioxidant, antidiabetic activity and hypolipidemic activity in STZ induced rats as compared to ACE at the dose studied.

Ruchi Shakya *et al* (2014).,[57] Formulated and characterized phytosome suspension of *Urtica dioica* (UD) . Extraction of *Urtica dioica* was done by maceration technique using ethanol (70%). Formulation of herbosome suspension was done by solvent evaporation technique. Phytosme suspension thus formed was evaluated for visualization, particle size, surface tension activity, stability, pH. Phytosome suspension appeared irregular spheres shape when seen through optical microscope and SEM. Particle size and charge determination of all formulation showed size in microns range and possess good stability. FTIR analysis and interpretation of all formulation reveals formation of complex and presence of hydrogen bonding, Short term stability studies on the most satisfactory formulation F5 was done as per ICH guideline showed no or little change in particle size, surface tension, and chemical integrity of suspension before and after stability studies. These findings suggest that herbosome suspension of *Urtica dioica* (100 mg/kg) shows better antidiabetic activity in comparisons to the powdered marketed formulations of *Urtica dioica*.

Literature Review About *Morinda citrifolia*

Saini Jasdeep Kaur *et al* (2016).,[58] Determined the anti-mycotic activity of commercially available Noni extract against *Malassezia furfur*, lipophilic yeast causing seborrhoeic dermatitis and other superficial skin infections. According to the anti-mycotic activity assay carried out using well diffusion method, the zone of an inhibition of Noni extract was compared to the zone of inhibition of few natural products and standard antidandruff shampoo. It was determined that Noni juice showed comparatively higher anti-mycotic activity, thus proving as an effective alternative against seborrhoeic dermatitis.

Afa K. Palu *et al* (2012).,[59] Evaluated the ability of noni extracts and preparations which inhibit MMP, COX-2 and Cat-G enzymes *in vitro*, as its mechanism of action for healing sun burn known as *fohia* in Tonga. Noni leaf ethanolic extract inhibited MMP-1, -2, -3, and -9 enzymes concentration dependently with 0.517, 0.234, 0.184, and 0.302 mg/mL IC50. Noni fruit juice concentrates in 1 and 5 mg/mL concentrations, inhibited MMP-12 enzymes by 102, and 99%, respectively. Both the extract and juice inhibited Cat-G enzymes concentration dependently with 0.125, <0.1, and 0.41 mg/mL IC50, respectively which help in the treatment of *fohia* skin . These results warrant further studies into the skin health benefits of noni fruit and leaf to further assess their efficacies and dosages in human subjects suffering from photoaging.

Praveen K. Ramamoorthy *et al.,(2007)*[60] Soxhlet, Ultrasonic and four extracts from high pressure extraction at 10 MPa using ethanol, ethyl acetate as solvent of *Morinda* citrifolia L.extract were analyzed for antioxidant activity by peroxide value method and diphenylpicrylhydrazyl radical scavenging method. Further five extracts were subjected to determine their total phenolic content by Folin-Ciocalteau method and total flavonoid content by Dowd method. The *M. citrifolia* extract by high pressure extraction was found to exhibit highest antioxidant activity and total flavonoid content. High total phenolic content was determined in the high pressure extract using ethyl acetate as solvent and vacuum dried. The ultrasonic extract exhibited significant antioxidant activity, total phenolic and flavonoid content. High pressure extracted *M. citrifolia* in ethanol was found to express lesser values comparatively. The significant difference in activity among the high pressure extracts was found to be due to the polarity of the solvents used for extraction as *M. citrifolia* fruit contains relatively larger quantity of non-polar antioxidant compounds. It was also found that the drying methods had significant impact on the antioxidant activity, total phenolic and flavonoid content of the extracts.

CH. Kethani Devi *et al* (2013).,[61] The petroleum ether and alcoholic extract of *Morinda citrifolia L.* extract were subjected to preliminary screening for Antimicrobial and Aanthelmintic activity. The alcoholic extract exhibited significant Anti-bacterial, Antifungal activity, comparable to the standard drug Tetracycline. The Petroleum Ether and Alcoholic extract were evaluated for Anthelmintic activity on adult Indian Earthworms, '*Pheretima posithuma'*. The Alcoholic extract produced more significant Anthelmintic activity than Petroleum ether extract and the activities are comparable with the reference drug Piperazine citrate.

Afa K. Palu *et al* (2012).,[62] Investigated whether extracts and preparations of noni fruit and leaf inhibit MMP, COX-2 and Cat-G enzymes *in vitro*, as its mechanism of action for healing sun-burn known as *fohia* in Tonga. Noni leaf ethanolic extract (NLEE) inhibited MMP-1, -2, -3, and -9 enzymes concentration dependently with 0.517, 0.234, 0.184, and 0.302 mg/mL IC50, respectively. Noni fruit juice concentrates (NFJC) in 1 and 5 mg/mL concentrations, inhibited MMP-12 enzymes by 102, and 99%, respectively. NFJC and NLEE inhibited Cat-G enzymes concentration-dependently with 0.125, <0.1, and 0.41 mg/mL IC50, respectively. Noni fruit juice fractions 4 and 6 inhibited COX-2 and Cat-G enzymes by 85 and 89%, and 89 and 78%, respectively. Additionally, the noni fruit puree and noni leaf has 1.91 mg/g and 5.77 mg/g of ursolic acid, respectively. NFJC, and NLEE inhibitory effects on MMP, COX-2 and Cat-G enzymes might help explain the traditional usage of the noni fruits and leaves for treatment of *fohia* skin as alluded to by Polynesian traditional healers. These results warrant further studies into the skin health benefits of noni fruit and leaf to further assess their efficacies and dosages in human subjects suffering from photoaging.

S. L. Kakad *et al* (2015).,[63] Evaluated the phytochemical, antibacterial and antifungal activities of leaf extract of *Morinda citrifolia* . The antibacterial activity was tested against gram positive bacteria *Bacillus subtilis*, *Escherichia coli, Pseudomonas fluroscence* and *Salmonella typhi* using disc diffusion method. Methanol extract showed highest zone of inhibition in *B. subtilis*. The antifungal activity was tested against *Aspergillus niger, Candida albicans* and *Daedalea flavida*. Methanol extract showed highest zone of inhibition in *A. niger*. The leaf extract noticed phytochemicals such as tannin, phenol, alkaloid, flavonoids, glycosides, steroids and terpenoids. Both the bacterial and fungal strains were exhibited significant inhibition. Phenol and anthraquinone activity was also performed.

Mairim Russo Serafini *et al* (2011).,[64] Determined the antioxidant, anti-inflammatory, antinociceptive,and antibacterial properties of the aqueous extract from *M. citrifolia* leaves (AEMC). Antioxidant activity was observed against lipid peroxidation, nitric oxide, and hydroxyl radicals. The antinociceptive effect of AEMC was observed in the acetic acid–induced writhing test at the higher dose. Moreover, AEMC significantly reduced the leukocyte migration in doses of 200 and 400 mg/kg and showed mild antibacterial activity. Together, the results suggest that properties of *M.citrifolia* leaf extract should be explored further in order to achieve newer tools for managing painful and inflammation conditions, including those related to oxidant states.

Hiroshi Okamoto (2012).,[65] Investigated the effect of *Morinda citrifolia* extract in the treatment of psoriasiss by a combination with conventional anti-rheumatism therapy by administering 4g/day of *Morinda citrifolia* (Noni) powder together with weekly methotrexate (MTX) . Psoriatic skin lesions surprisingly improved significantly . This is the first report showing the possibility of a clinical effect of *M. citrifolia* on psoriasis .Therefore Noni, together with MTX might have unknown immune-modulation effects on skin lesions and arthropathy associated with psoriasis.This case also gives us a clue to differentiate between skin lesions and arthropathy in terms of their pathogenesis.

Brett J. West *et al* (2012).,[66] A six week clinical trial of a *Morinda citrifolia* (noni) based skin care regimen was conducted with 49 women, ages 38 to 55 years. Daily application of three product formulations to the face and neck resulted in significant reductions in lateral canthal fine lines and wrinkles (crow's feet), as measured by technician scoring and digital image analysis. Use of the regimen also improved skin elasticity. No evidence of skin irritation was present in any participant at any time during the trial. A study questionnaire revealed that the measured improvements were visibly perceptible to more than 90% of the participants. The trial results substantiate traditional uses of the noni plant to improve skin health.

AIM AND OBJECTIVES

Morinda citrifolia L. (Rubiaceae), commonly known as Noni, has been extensively used in folk medicine by the Polynesians for over 2000 years. It is having a high demand as an alternative medicine due to its use as antioxidant, anti fungal , anti bacterial, anti-inflammatory, liver protective, anticancer, analgesic, immunomodulatory, anti viral and wound healing effects etc. Phytochemical investigations have shown that noni contains a number of phenolic compounds, in particular, coumarins , flavonoids, and iridoids which are reported to have various pharmacological effects .

However, these phytoconstituents are poorly absorbed either due to their large molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability.

Phytosomes are vesicular drug delivery systems which incorporate plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes. They provide better absorption and bioavailability than the conventional herbal extracts.

The aim of the present study was to prepare and evaluate topical phytosomal gel of *Woodfordia fruticosa* with an objective to increase its bioavailability and therapeutic efficacy.

The objectives of the study are:

- To formulate phytosomes containing *Morinda citrifolia* extract
- To characterize the prepared phytosomes using various methods like , entrapment efficiency , percentage yield , solubility, drug content , *in vitro* drug diffusion studies , *ex-vivo* permeation study vesicular shape, and physical stability of phytosomes,
- To prepare phytosomal gel containing *Morinda citrifolia* extract
- To carry out pH, spreadability coefficient, extrudability, drug content, rheological studies and *in-vitro* drug diffusion study and drug release kinetic data analysis of the optimized formulation of phytosomal gel containing *Morinda citrifolia* extract.

- To carry out the stability studies on the optimized formulation of phytosomes as per ICH guidelines.

PLAN OF WORK

1. Selection of plant drug .

2. Initial phyto chemical screening of the plant extract .

3. Determination of λ_{max}.

4. Construction of standard calibration curve of *Morinda citrifolia extract*

5. Formulation of phytosomes containing *Morinda citrifolia* extract.

6. Evaluation of different physicochemical parameters of ethosomes.

- Entrapment efficiency
- Percentage drug content
- Percentage yield
- Vesicle shape (SEM)
- *In vitro* dissolution studies
- *Ex-vivo* permeation study

7. Preparation of. phytosomal gel containing *Morinda citrifolia* extract

8. Evaluation of phytosomal gel.

- pH
- Spreadability coefficient
- Extrudability.
- Viscosity
- Drug content
- *In vitro* diffusion study

9. Carry out drug release kinetic data analysis and stability studies of the optimised formulation of phytosomes as per ICH guidelines

PLANT PROFILE

Morinda citrifolia :[28-30]

❖ **BOTANICAL DESCRIPTION**

The botanical name for the genus was derived from the two Latin words *morus*, mulberry, and *indicus*, Indian, in reference to the similarity of the fruit of noni to that of true mulberry (*Morus alba*). The species name indicates the resemblance of the plant foliage to that of some citrus species.

❖ **Family**

Rubiaceae (coffee family)

❖ **Plant Parts**

• **Fruits**

Figure no: 4

The fruit (technically known as a syncarp) is yellowish white, fleshy, 5–10 cm (2–4 in) long, about 3–4 cm (1.2–1.6 in) in diameter, and soft and fetid when ripe.

• **Flowers**

Figure no:5

Flowers are perfect, with about 75–90 in ovoid to globose heads. Peduncles are 10–30 mm

(0.4– 1.2 in) long, the calyx a truncated rim. The corolla is white, 5–lobed, the tube greenish white, 7–9 mm (0.28–0.35 in) long, the lobes oblong- deltate, approximately 7 mm (0.28 in) long. There are five stamens, scarcely exserted; the style is about 15 mm (0.7 in) long.

• **Leaves**

Figure no:6

Leaves are opposite, pinnately veined, and glossy. Blades are membraneous, elliptic to ellipticovate, 20–45 cm (8–18 in) long, 7–25 cm (3.5–10 in) wide, and glabrous. Petioles are stout, 1.5–2 cm (0.6– 0.8 in) long. Stipules are connate or distinct, 1–1.2 cm (0.4–0.5 in) long, the apex entire or 2–3-lobed.

• **Seeds**

Figure no:7

Seeds have a distinct air chamber, and can retain viability even after floating in water for months.

❖ **Taxonomical Classification**

Botanical Name	*Morinda citrifolia*
Family	Rubiaceae
Kingdom	Plantae
Subkingdom	Rubioideae
Class	Magnoliopsida
Phylum	Magnoliophyta
Order	Rubiales
Genus	Morinda
Species	citrifolia

Table no:1

❖ **Regional Names**

Hindi	Baratundi
Telungu	Mogali
Marathi	Nagakunda
Tamil	Nuna
Malayalam	Manavapatta
Kannada	Tagase
Gujarati	Surangi
Bengali	Hurdi

Table no:2

❖ **Active constituents**

About 160 phytochemicals have been identified in the noni plant, including phenolic compounds, flavonoids , organic acids, and alkaloids. Among the phenolic compounds found, the most important are anthraquinones (eg, damnacanthal, morindone, morindin, aucubin, asperuloside, and scopoletin). The main organic acids are caproic and caprylic acids, while the principal reported alkaloid is xeronine.

Minerals account for 8.4% of the dry matter and are mainly potassium, sulfur, calcium, and phosphorus, with traces of selenium . Vitamins have been identified in the fruit, mainly ascorbic acid (24 to 158 mg per 100 g dry matter), and provitamin A.

Phenolic compounds are the major group of compounds which are scopoletin, morindone, alizarin, aucubin, nordamnacanthal, rubiadin, rubiadin-1-methyl ether, and other anthraquinone glycosides have been identified. Damnacanthal is an anthraquinone that has been characterized and may have anticarcinogenic properties. Scopoletin is a coumarin with analgesic properties as well as an ability to control serotonin levels in the body. Other studies have shown that scopoletin may also have antimicrobial and antihypertensive effects.

Another noni component, proxeronine, is the precursor of xeronine, an alkaloid that is claimed to combine with human proteins, improving their functionality. About 51 volatile compounds have been identified in the ripe fruit, including organic acids (mainly octanoic and hexanoic acids), alcohols (3-methyl-3-buten-1-ol), esters (methyl octanoate, methyl decanoate), ketones (2-heptanone),

The potassium content is relatively high (30 to 50 ppm), followed by calcium, sodium, and magnesium. Vitamin C content varies from 30 to 155 mg/kg. The polysaccharide fraction consists primarily of the pectins homogalacturonan, rhamnogalacturonan I, arabinan, and type I and II arabinogalactans.

Numerous iridoids, with the main compounds asperuloside, asperulosidic acid, and deacetylasperulosidic acid . Minor iridoids include deacetylasperuloside,

dehydromethoxygaertneroside, epi -dihydrocornin, 6-alpha-hydroxyadoxoside, citrifolinin B epimers a and b, and 7-beta-epoxy-8- epi –splendoside

Flavonol glycosides include rutin, narcissoside, and nicotifloroside. Several known and new lignans, such as 3,3′-bisdemethylpinoresinol, americanol A, americanin A, americanoic acid A, morindolin, isoprincepin, and balanophonin, have been isolated. The coumarin scopoletin has also been identified. Similar to other plant parts, the contain a wide spectrum of 1-hydroxyanthraquinones, albeit in much lower concentrations. These include novel compounds, such as 2-methoxy-1,3,6-trihydroxyanthraquinone and 5,15-dimethylmorindol. Finally, miscellaneous compounds, such as J-sitosterol and its 3- O -glucoside, ursolic acid, and 19-hydroxyursolic acid, cytidine, borreriagenin, and epiborreriagenin, iridoidderivative, succinic acid diesters, 4-hydroxy-3-methoxycinnamaldehyde, J-hydroxypropiovanillone, and vanillin have been isolated.

Some essential oils with hexanoic and octanoic acids, paraffin, and esters of ethyl and methyl alcohols. Ripe fruits contain n-caproic acid, presumably responsible for the distinctive odor, known to attract insects such as Drosophilia sechellia . Fresh plants contain anthraquinones, morindone, and alizarin.

- **Dosing**

30 to 750 mL/day; dosing of 500 mg extract is nontoxic. The appropriate dose of noni depends on several factors such as the user's age, health, and several other conditions. At this time there is not enough scientific information to determine an appropriate range of doses for noni. Keep in mind that natural products are not always necessarily safe and dosages can be important. Be sure to follow relevant directions on product labels and consult your pharmacist or physician or other healthcare professional before using.

- **Therapeutic Uses**

Noni is one of the most frequently used Hawaiian plant medicines. All plant parts are used for a variety of illnesses which include , anti oxidant , anti inflammatory , anti-arthritic , antibacterial, antifungal , anticancer , anticoagulant , antivirus , antispasmodic , immunomodulatory , wound-healing , neuroprotective , treatment for burns , sore or irritated eyes, styes, conjunctivitis, ocular inflammation , arthritic pain

- **Toxicology**

Some medications for high blood pressure like ACE inhibitors , Angiotensin receptor blockers can increase potassium levels in the blood. Medications that can harm the liver can increase the risk of liver damage. Do not take noni if you are taking a medication that can harm the liver. Warfarin (Coumadin) is used to slow blood clotting. Taking noni might decrease how well warfarin (Coumadin) works to slow blood clotting. This could increase the chance of blood clotting..Noni contains large amounts of potassium. Some "water pills" can also increase potassium levels in the body. Taking some "water pills" along with noni might cause too much potassium to be in the body. Some "water pills" that increase potassium in the body include amiloride (Midamor), spironolactone (Aldactone), and triamterene (Dyrenium).

- **Special Precautions & Warnings:**

Pregnancy and breast-feeding :. Historically, noni has been used to cause abortions. It is also best to avoid noni if you are breast-feeding. Not enough is known about the safety of taking noni during breast-feeding.

Kidney problems : Noni contains large amounts of potassium. This can be a problem, especially for people with kidney disease. There is one report of a person with kidney disease developing high levels of potassium in the blood after drinking noni juice. Don't use noni if you have kidney problems.

High potassium levels : Drinking noni fruit juice might increase potassium levels and make them even higher in people with already too much potassium in their body. **Liver disease** : Noni has been linked to several cases of liver damage. Avoid using noni if you have liver disease

EXCIPIENT PROFILE[32-38]

SOYA LECITHIN

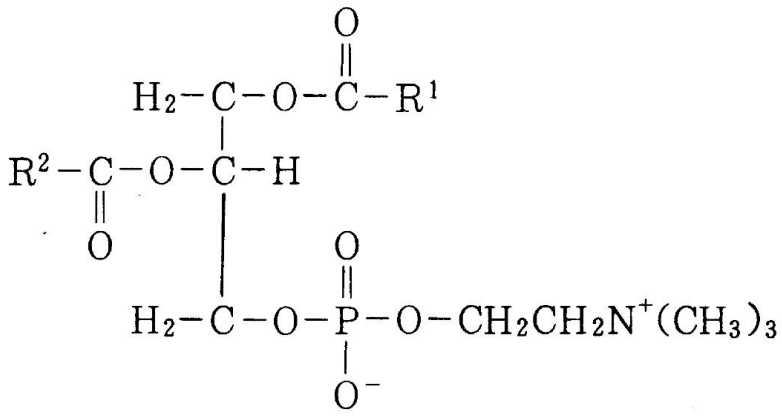
- **SYNONYM**

  Mixed soybean phosphatides, Ovolecithin, Phospholipon, Soybean phospholipids

- **CHEMICAL NAME**

  1,2-diacyl-sn-glycero-3-phosphocholine

- **CHEMICAL STRUCTURE**



- **CHEMICAL COMPOSITION**

  Contains 21% phosphatidylcholine, 22% phosphatidylethanolamine and 19% phosphatidylinositol, along with other components

- **DESCRIPTION**

  Lecithins vary greatly in their physical form, from viscous semi liquids to powders, depending upon the free fatty acid content. They may also vary in colour from brown to light yellow, depending upon whether they are bleached or unbleached or on the degree of purity.

- **FUNCTIONAL CATEGORY**

  Emollient, emulsifying agent and solubilising agent

- **SOLUBILITY**

  Soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids. Sparingly soluble in ethanol (95%). They are practically insoluble in cold

vegetable and animal oils, polar solvents, and water. When mixed with water, however, lecithins hydrate to form emulsions.

- **DENSITY**

  0.5 g/cm^3

- **ISOELECTRIC POINT**

  3.5

- **COMPATIBILITY**

  Incompatible with esterases owing to hydrolysis

- **STABILITY**

  Lecithins decompose at extreme pH. They are also hygroscopic and subject to microbial degradation. When heated, lecithins oxidize, darken, and decompose. Temperatures of 160–180^{o}C will cause degradation within 24 hours.

- **FLAMMABILITY**

  Low

- **MOISTURE CONTENT**

  Are hygroscopic

- **SPECIFIC GRAVITY**

  0.97 g/cm^3

- **SAFETY**

  Is non-toxic, but excessive consumption may be harmful.

- **STORAGE**

  Should be stored in well-closed containers protected from light, moisture and oxidation. Purified solid lecithins should be stored in tightly closed containers at subfreezing temperatures

- **APPLICATIONS**

  - Lecithins are used in a wide variety of pharmaceutical applications like aerosol inhalations, IM injections and oral suspension and are also used in cosmetics and food products.
  - Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents, and are included in intramuscular and

intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments.

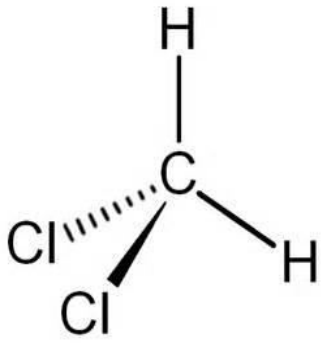
- Lecithins are also used in suppository bases to reduce the brittleness of suppositories, and have been investigated for their absorption-enhancing properties in an intranasal insulin formulation.
- Lecithins are also commonly used as a component of enteral and parenteral nutrition formulations. Lecithin is a required component of FDA-approved infant formulas.
- Therapeutically, lecithin and derivatives have been used as a pulmonary surfactant in the treatment of neonatal respiratory distress syndrome

DICHLOROMETHANE

- **SYNONYM**

  Methyelene chloride , Solmethine , Narkotil , Solaesthin , Refrigerant-30- Freon -30

- **CHEMICAL STRUCTURE**



- **MOLECULAR FORMULA**

  CH_2Cl_2

- **MOLAR MASS**

  84.93 $g.mol^{-1}$

- **APPEARANCE**

  Colourless liquid

- **ODOR**

  Chloroform like

- **DENSITY**

  1.3266 g/cm^3

- **VISCOSITY**

  0.437mPa.s at 20˚C

- **MELTING POINT**

  -96.7˚C

- **BOLING POINT**

  39.6˚C

- **SOUBILITY**

  Soluble in water and miscible in ethyl acetate , alcohol , hexanes , benzene , diethyl ether

- **STORAGE**

  Store in well- closed container in a cool, dry place.

- **APPLICATION**

  - Its volatility and ability to dissolve a wide range of organic compounds makes it a useful solvent for many chemical processes.
  - It is widely used as a paint stripper and a degreaser.
  - In the food industry, it has been used to decaffeinate coffee and tea as well as to prepare extracts of hops and other flavorings.
  - Its volatility has led to its use as an aerosol spray propellant and as a blowing agent for polyurethane foams.
  - Used as a process solvent in the manufacture of pharmaceuticals .
  - Used as an extraction solvent for spice oleoresin, removal of caffeine from coffee.
  - It is also used as a post harvest fumigant for grains.

ETHANOL

- **SYNONYM**

  Absolute alcohol, Ethyl alcohol , Grain alcohol, Methylcarbinol, Anhydrol, Cologne spirit, Ethylic alcohol

- **CHEMICAL NAME**

  Ethanol

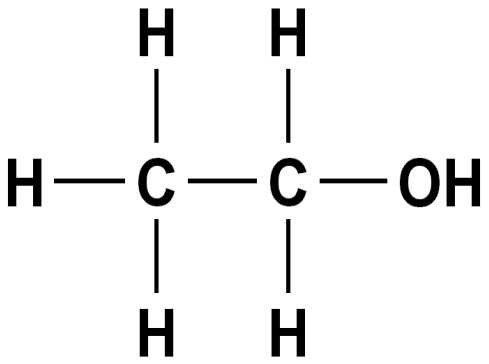
- **CHEMICAL FORMULA**

  C_2H_6O

- **MOLECULAR WEIGHT**

  $46.07 g.mol^{-1}$

- **CHEMICAL STRUCTURE**



- **APPEARANCE**

  Colorless liquid

- **ODOR**

  Colorless liquid with weak, ethereal, vinous odor

- **TASTE**

  Burning

- **BOILING POINT**

  78.29˚C

- **MELTING POINT**

  -114.14˚C

- **SOLUBILITY**

  Miscible with ethyl ether, acetone, chloroform and soluble in benzene.

- **DENSITY**

  0.7893 g/cu cm

- **SURFACE TENSION**

  21.97 mN/m

- **IONIZATION POTENTIAL**

  10.47 eV

- **pKa**

  15.9

- **STORAGE**

  Store in well- closed container in a cool, dry place.

- **APPLICATIONS**

  - Ethanol is used in medical wipes and most common antibacterial hand sanitizer gels as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria and fungi, and many viruses. However, ethanol is ineffective against bacterial spores.
  - Ethanol may be administered as an antidote to methanol[23] and ethylene glycol poisoning.
  - used to dissolve many water-insoluble medications and related compounds.
  - As a central nervous system depressant, ethanol is one of the most commonly consumed psychoactive drugs.
  - The largest single use of ethanol is as an engine fuel and fuel additive..
  - Ethanol is miscible with water and is a good general purpose solvent. It is found in paints, tinctures, markers, and personal care products such as mouthwashes, perfumes and deodorants.

CARBOPOL 934

- **SYNONYMS**

  Acrypol, acryli acid polymer, carbomer, poly acrylic acid, carboxyvinyl polymer, carboxy poly methylene, tego carbomer

- **NON-PROPRIETRY NAME**

  BP : Carbomer

  PhEur: Carbomers

  USP-NF: Carbomers

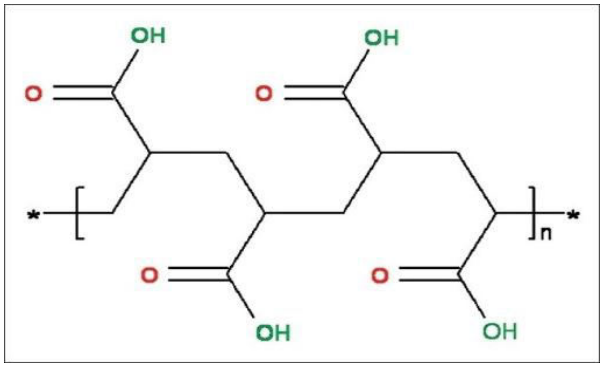
- **CHEMICAL NAME**

  Polyacrylate – 1 – cross polymer

- **MOLECULAR WEIGHT**

  Approx. 500,000 to 4,000,000. g

- **CHEMICAL STRUCTURE**



- **COLOUR**

  White, light, acidic, hygroscopic powder.

- **PARTICLE SIZE**

  Flocculated powder having a median diameter of 2 to 7 microns.

- **TOTAL SOLID**

  100%

- **DENSITY**

  Approximately208kg/m^2

- **SPECIFIC GRAVITY**

  1.41

- **MOISTURE CONTENT**

  2.0% maximum

- **VISCOSITY**

  Between 30500 and 39400 centipoise

- **SOLUBILITY**

  They are insoluble due to their cross linked nature and high molecular weight.

- **pKa**

  6.0

- **SWELLING PROPERTIES**

  They get swell in water and some polar solvents, producing viscous dispersions .

- **WETTING TIME**

  3 minute

- **PACKING AND STORAGE**

  Preserve in tight containers

- **ADVANTAGES**

  - Good rheological properties on the application site.
  - Substitute to oil-based ointment formulations
  - Viscosity is high at low concentration
  - Compatibility
  - Better bioadhesive properties
  - Good thermal stability

- **APPLICATION**

  - Very well suited to aqueous formulations of the topical dosage forms such as hydrogel. Many commercial topical products available today have been formulated with these polymers. They provide the following plentiful advantage to topical formulations
  - Long antiquity of safe and effective use in topical gels, creams and ointments. They are also supported by board toxicology studies .
  - Good tablet flow property.

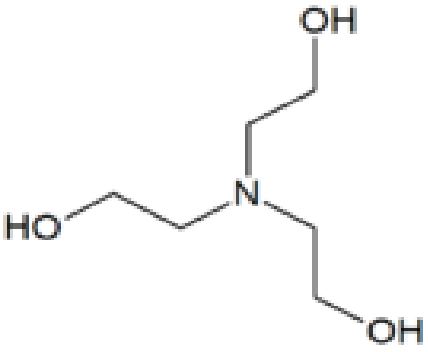
- Shows extremely low irritancy properties and are non-sensitizing with repeat usage.
- Long drug release.
- Provides a magnificent vehicle for drug delivery. Due to their high molecular weight, they are not able to penetrate the skin or affect the therapeutic efficacy of the drug.
- Superior thickening, suspending, & emulsification properties for topical preparations. Products with a wide range of viscosities and flow properties have been successfully formulated and commercialized.

TRIETHANOLAMINE

- **SYNONYMS**

  Tris(2-hydroxyethyl)amine, Triethylolamine, Trolamine, 2,2,2" -Trihydroxytriethylamine

- **CHEMICAL STRUCTURE**



- **CHEMICAL NAME**

  2,2,2" -Nitrilotri(ethan-1-ol)

- **CHEMICAL FORMULA**

  $C_6H_{15}NO_3$

- **MOLECULAR WEIGHT**

  149..19 g.mol

- **APPERANCE**

  Coloress viscous hygroscopic liquid or crystals with characteristics odor

- **ODOR**

  Ammonical

- **pH**

  10.5 Strong base

- **DENSITY**

  1.124 g mL^{-1}

- **VISCOSITY**

  65.7 cP

- **SOLUBILITY**

  Miscible with water, methanol, acetone soluble in benzene, ether, carbon tetra chloride, n-heptane

- **MELTING POINT**

  21.60˚C

- **BOILING POINT**

  333.40˚C

- **VAPOR PRESSURE**

  1 Pa (20˚C)

- **PKa**

  7.74

- **PACKING AND STORAGE**

  Preserve in tight containers

- **APPLICATIONS**

  - Triethanolamine is used primarily as an emulsifier and surfactant.
  - The triethanolamine neutralizes fatty acids, adjusts and buffers the pH, and solubilizes oils and other ingredients that are not completely soluble in water.
  - Some common products in which triethanolamine is found are liquid laundry detergents, dishwashing liquids, general cleaners, hand sanitizers, polishes and organic additive (0.1 wt%) in the grinding of cement clinker.
  - It is an active ingredient of some eardrops used to treat impacted earwax.
  - It also serves as a pH balancer in many different cosmetic products, ranging from cleansing creams and milks, skin lotions, eye gels, moisturizers, shampoos, shaving foams, and so on.

- Used as a complexing agent for aluminium ions in aqueous solutions.
- 2-3% in water triethanolamine is used as an corrosion inhibitor (anti-rust) agent in immersion ultrasonic testing.

EXCIPIENT PROFILE[32-38]

SOYA LECITHIN

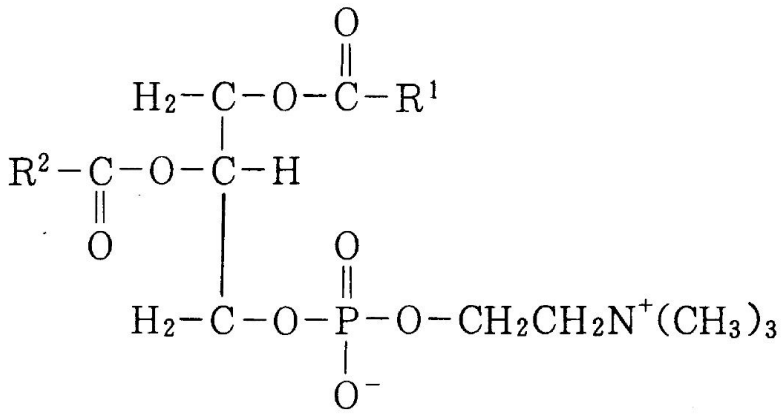
- **SYNONYM**

  Mixed soybean phosphatides, Ovolecithin, Phospholipon, Soybean phospholipids

- **CHEMICAL NAME**

  1,2-diacyl-sn-glycero-3-phosphocholine

- **CHEMICAL STRUCTURE**



- **CHEMICAL COMPOSITION**

  Contains 21% phosphatidylcholine, 22% phosphatidylethanolamine and 19% phosphatidylinositol, along with other components

- **DESCRIPTION**

  Lecithins vary greatly in their physical form, from viscous semi liquids to powders, depending upon the free fatty acid content. They may also vary in colour from brown to light yellow, depending upon whether they are bleached or unbleached or on the degree of purity.

- **FUNCTIONAL CATEGORY**

  Emollient, emulsifying agent and solubilising agent

- **SOLUBILITY**

  Soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids. Sparingly soluble in ethanol (95%). They are practically insoluble in cold

vegetable and animal oils, polar solvents, and water. When mixed with water, however, lecithins hydrate to form emulsions.

- **DENSITY**

  0.5 g/cm^3

- **ISOELECTRIC POINT**

  3.5

- **COMPATIBILITY**

  Incompatible with esterases owing to hydrolysis

- **STABILITY**

  Lecithins decompose at extreme pH. They are also hygroscopic and subject to microbial degradation. When heated, lecithins oxidize, darken, and decompose. Temperatures of 160–180^{o}C will cause degradation within 24 hours.

- **FLAMMABILITY**

  Low

- **MOISTURE CONTENT**

  Are hygroscopic

- **SPECIFIC GRAVITY**

  0.97 g/cm^3

- **SAFETY**

  Is non-toxic, but excessive consumption may be harmful.

- **STORAGE**

  Should be stored in well-closed containers protected from light, moisture and oxidation. Purified solid lecithins should be stored in tightly closed containers at subfreezing temperatures

- **APPLICATIONS**

  - Lecithins are used in a wide variety of pharmaceutical applications like aerosol inhalations, IM injections and oral suspension and are also used in cosmetics and food products.
  - Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents, and are included in intramuscular and

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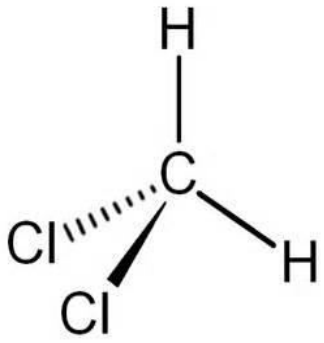
- Therapeutically, lecithin and derivatives have been used as a pulmonary surfactant in the treatment of neonatal respiratory distress syndrome

DICHLOROMETHANE

- **SYNONYM**

  Methyelene chloride , Solmethine , Narkotil , Solaesthin , Refrigerant-30- Freon -30

- **CHEMICAL STRUCTURE**



- **MOLECULAR FORMULA**

  CH_2Cl_2

- **MOLAR MASS**

  84.93 $g.mol^{-1}$

- **APPEARANCE**

  Colourless liquid

- **ODOR**

  Chloroform like

- **DENSITY**

  1.3266 g/cm^3

- **VISCOSITY**

  0.437mPa.s at 20˚C

- **MELTING POINT**

  -96.7˚C

- **BOLING POINT**

  39.6˚C

- **SOUBILITY**

  Soluble in water and miscible in ethyl acetate , alcohol , hexanes , benzene , diethyl ether

- **STORAGE**

  Store in well- closed container in a cool, dry place.

- **APPLICATION**

  - Its volatility and ability to dissolve a wide range of organic compounds makes it a useful solvent for many chemical processes.
  - It is widely used as a paint stripper and a degreaser.
  - In the food industry, it has been used to decaffeinate coffee and tea as well as to prepare extracts of hops and other flavorings.
  - Its volatility has led to its use as an aerosol spray propellant and as a blowing agent for polyurethane foams.
  - Used as a process solvent in the manufacture of pharmaceuticals .
  - Used as an extraction solvent for spice oleoresin, removal of caffeine from coffee.
  - It is also used as a post harvest fumigant for grains.

ETHANOL

- **SYNONYM**

  Absolute alcohol, Ethyl alcohol , Grain alcohol, Methylcarbinol, Anhydrol, Cologne spirit, Ethylic alcohol

- **CHEMICAL NAME**

  Ethanol

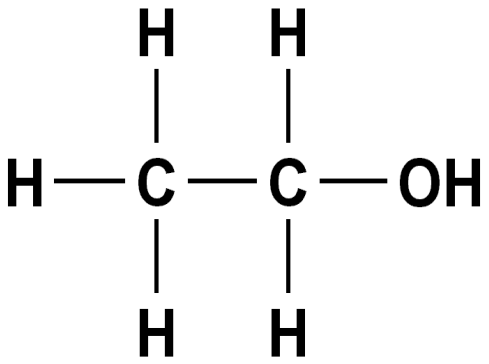
- **CHEMICAL FORMULA**

  C_2H_6O

- **MOLECULAR WEIGHT**

  $46.07 g.mol^{-1}$

- **CHEMICAL STRUCTURE**



- **APPEARANCE**

  Colorless liquid

- **ODOR**

  Colorless liquid with weak, ethereal, vinous odor

- **TASTE**

  Burning

- **BOILING POINT**

  78.29˚C

- **MELTING POINT**

  -114.14˚C

- **SOLUBILITY**

  Miscible with ethyl ether, acetone, chloroform and soluble in benzene.

- **DENSITY**

  0.7893 g/cu cm

- **SURFACE TENSION**

  21.97 mN/m

- **IONIZATION POTENTIAL**

  10.47 eV

- **pKa**

  15.9

- **STORAGE**

  Store in well- closed container in a cool, dry place.

- **APPLICATIONS**

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  - Ethanol may be administered as an antidote to methanol[23] and ethylene glycol poisoning.
  - used to dissolve many water-insoluble medications and related compounds.
  - As a central nervous system depressant, ethanol is one of the most commonly consumed psychoactive drugs.
  - The largest single use of ethanol is as an engine fuel and fuel additive..
  - Ethanol is miscible with water and is a good general purpose solvent. It is found in paints, tinctures, markers, and personal care products such as mouthwashes, perfumes and deodorants.

CARBOPOL 934

- **SYNONYMS**

  Acrypol, acryli acid polymer, carbomer, poly acrylic acid, carboxyvinyl polymer, carboxy poly methylene, tego carbomer

- **NON-PROPRIETRY NAME**

  BP : Carbomer

  PhEur: Carbomers

  USP-NF: Carbomers

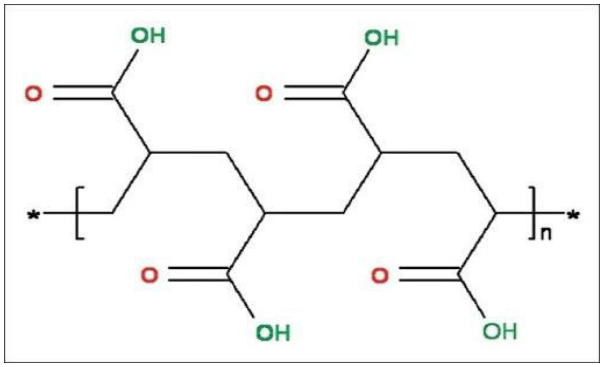
- **CHEMICAL NAME**

  Polyacrylate – 1 – cross polymer

- **MOLECULAR WEIGHT**

  Approx. 500,000 to 4,000,000. g

- **CHEMICAL STRUCTURE**



- **COLOUR**

  White, light, acidic, hygroscopic powder.

- **PARTICLE SIZE**

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- **TOTAL SOLID**

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- **DENSITY**

  Approximately208kg/m^2

- **SPECIFIC GRAVITY**

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- **MOISTURE CONTENT**

  2.0% maximum

- **VISCOSITY**

  Between 30500 and 39400 centipoise

- **SOLUBILITY**

  They are insoluble due to their cross linked nature and high molecular weight.

- **pKa**

  6.0

- **SWELLING PROPERTIES**

  They get swell in water and some polar solvents, producing viscous dispersions .

- **WETTING TIME**

  3 minute

- **PACKING AND STORAGE**

  Preserve in tight containers

- **ADVANTAGES**

  - Good rheological properties on the application site.
  - Substitute to oil-based ointment formulations
  - Viscosity is high at low concentration
  - Compatibility
  - Better bioadhesive properties
  - Good thermal stability

- **APPLICATION**

  - Very well suited to aqueous formulations of the topical dosage forms such as hydrogel. Many commercial topical products available today have been formulated with these polymers. They provide the following plentiful advantage to topical formulations
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  - Good tablet flow property.

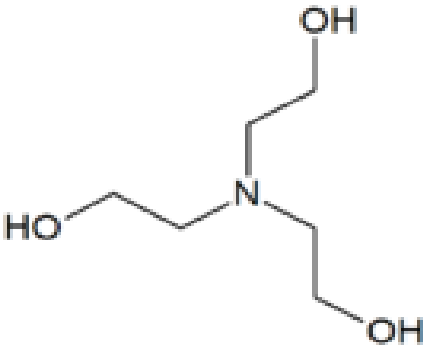
- Shows extremely low irritancy properties and are non-sensitizing with repeat usage.
- Long drug release.
- Provides a magnificent vehicle for drug delivery. Due to their high molecular weight, they are not able to penetrate the skin or affect the therapeutic efficacy of the drug.
- Superior thickening, suspending, & emulsification properties for topical preparations. Products with a wide range of viscosities and flow properties have been successfully formulated and commercialized.

TRIETHANOLAMINE

- **SYNONYMS**

  Tris(2-hydroxyethyl)amine, Triethylolamine, Trolamine, 2,2,2" -Trihydroxytriethylamine

- **CHEMICAL STRUCTURE**



- **CHEMICAL NAME**

  2,2,2" -Nitrilotri(ethan-1-ol)

- **CHEMICAL FORMULA**

  $C_6H_{15}NO_3$

- **MOLECULAR WEIGHT**

  149..19 g.mol

- **APPERANCE**

  Coloress viscous hygroscopic liquid or crystals with characteristics odor

- **ODOR**

  Ammonical

- **pH**

  10.5 Strong base

- **DENSITY**

  1.124 g mL^{-1}

- **VISCOSITY**

  65.7 cP

- **SOLUBILITY**

  Miscible with water, methanol, acetone soluble in benzene, ether, carbon tetra chloride, n-heptane

- **MELTING POINT**

  21.60˚C

- **BOILING POINT**

  333.40˚C

- **VAPOR PRESSURE**

  1 Pa (20˚C)

- **PKa**

  7.74

- **PACKING AND STORAGE**

  Preserve in tight containers

- **APPLICATIONS**

  - Triethanolamine is used primarily as an emulsifier and surfactant.
  - The triethanolamine neutralizes fatty acids, adjusts and buffers the pH, and solubilizes oils and other ingredients that are not completely soluble in water.
  - Some common products in which triethanolamine is found are liquid laundry detergents, dishwashing liquids, general cleaners, hand sanitizers, polishes and organic additive (0.1 wt%) in the grinding of cement clinker.
  - It is an active ingredient of some eardrops used to treat impacted earwax.
  - It also serves as a pH balancer in many different cosmetic products, ranging from cleansing creams and milks, skin lotions, eye gels, moisturizers, shampoos, shaving foams, and so on.

- Used as a complexing agent for aluminium ions in aqueous solutions.
- 2-3% in water triethanolamine is used as an corrosion inhibitor (anti-rust) agent in immersion ultrasonic testing.

MATERIALS AND METHODS

Table 3: LIST OF EXCIPIENTS USED AND THEIR SOURCES

Sl.No	MATERIAL	SOURCES
1	Soya lecithin	HiMedia Laboratories, Nashik
2	Dichloromethane	Loba Chemie, Mumbai
3	Ethanol	Changshu Yangyuan Chemical China
4	Hydrochloric acid	SD Fine Chem Ltd, Mumbai
5	Carbopol 934	Loba Chemie, Mumbai
6	Triethanolamine	SD fine chemicals , Mummbai
7	Propyl Paraben	HiMedia Laboratories, Nashik

Table 4: LIST OF EQUIPMENTS USED

Sl.No	EQUIPMENTS	SUPPLIERS / MANUFACTURES
1	Rotary vaccum evaporator	Superfit, India
2	Weighing balance	Shimadzu, Shimadzu corporation, Philippines
3	Heating mantle	Sunbim, India
4	Viscometer	Brookfield Viscometer , USA
5	Sonicator	Life Care
6	Dissolution apparatus	Tab machines, Mumbai, India
7	Magnetic Stirrer	Remi ,Mumbai
8	Centrifuge Apparatus	Remi , Mumbai
9	Stability chamber	Technico, Chennai, India
10	Digital pH meter	EUTECH Instruments, India
11	Scanning electron microscope	JEOL, Japan

PREPARATION OF PLANT EXTRACT[31]

The leaves of plant was air-dried until dryness at room temperature and under shade. The dried leaves was then powdered to a fine grade by using laboratory scale mill. Further it was sequentially extracted successively with ethanol using soxhlet apparatus. The solvent was removed and concentrated in a rotary evaporator and water bath. The dried extracts were stored in refrigerator for further studies.[40,43]

DETERMINATION OF λ_{max}

The stock solution of 1000µg/ml was prepared by dissolving approximately100mg of pure *Morinda citrifolia* extract in 100ml of pH 7.4 phosphate buffer. From the stock solution, 10ml was taken and was further diluted to 100ml with the buffer solution. The prepared solution was then scanned in a wavelength range of 200-400nm, to find the maximum absorbance. The maximum wavelength was found to be 279nm and was used for further studies.

DETERMINATION OF STANDARD CURVE

The serially diluted stock solution was obtained in the range of 2- 10µg/ml by taking 0.2, 0.4, 0.6, 0.8 and 1 ml from the stock solution, into 100ml volumetric flask.

- The final solution is made by using phosphate buffer of pH 7.4.
- The serially diluted solutions were measured in a UV spectrometer at 279nm of the drug.
- The calibration curve was plotted by taking absorbance on Y-axis and concentration in µg/ml on X-axis, to find the slope.

FORMULATION OF PHYTOSOMES OF *Morinda citrifolia* EXTRACT BY ANTISOLVENT PRECIPITATION TECHNIQUE[42]

To prepare the phytosomes of *Morinda citrifolia* extract , drug extract and soya lecithin at molar ratio of 1:1, 1:2 ,1:3 , 1:4 , 1:5 , 1:6 and 1:7 were taken in the flask of vacuum rotary evaporator. Dichloromethane were added in the flask. The mixture was shaken at a temperature not exceeding 40˚C for 2 hours. The resultant solution was evaporated by increasing temperature up to 60˚C and by using vacuum pump in vacuum rotary evaporator. Ethanol was added to the flask with continuous stirring. The phytosomes was precipitated and ethanol was evaporated under vacuum to remove the traces of solvent. The dried residues were gathered and placed desiccators

over night, than crushed in the mortar and sieved through 80 mesh then subjected to further characterization.[34]

Table 5: Formulation table of *Morinda citrifolia* phytosome

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7
***Morinda citrifolia* : Soyalecithin**	1:1	1:2	1:3	1:4	1:5	1:6	1:7
Dichloromethane (ml)	20	20	20	20	20	20	20
Ethanol (ml)	5	5	5	5	5	5	5

EVALUATION OF PHYTOSOMAL COMPLEX

1 . Microscopic view[55]

Optical microscopy was used for characterization of the complex. The complex was suspended in buffer and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 45X.

2. Percentage Practical Yield[43]

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. Phytosomes prepared were collected and weighed to determine practical yield from the following equation:

$$(\%)\ \text{Yield} = \frac{(\text{Practical yield}) \times 100}{(\text{Theoretical yield})} \qquad \textbf{.....(1)}$$

3. Entrapment efficiency[41]

100 mg of *Morinda citrifolia* phytosomal complex were centrifuged at 2000rpm for 30 min using a Remi centrifuge to separate phytosomes from un entrapped drug. Concentration of the free

drug as the supernatant was determined by measuring absorbance at 279nm using UV-Visible spectrophotometer. The percentage drug entrapment was calculated by using the formula,

$$\text{Entrapment efficiency (\%)} = \frac{\text{(Total amount of drug)} - \text{(amount of free drug)}}{\text{(Total amount of drug}} \times 100 \quad \text{.....(2)}$$

4. Drug content[55]

Phytosomes equivalent to 10 mg of drug was accurately weighed and taken into a 100 ml volumetric flask. The contents of the flask was dissolved in small quantity of ethanol and sonicated for 30 minute. Volume was adjusted to 100 ml with ethanol. Contents of the flask were filtered and drug content was determined spectrophotometrically using UV spectrophotometer after appropriate dilutions.

5. Solubility Determination[55]

To determine the change in solubility due to complexation, the apparent solubility of drug extract and phytosomal complex was determined by adding an excess amount of drug and phytosomes to 6 ml distilled water, 7.4 pH phosphate buffer and n-octanol in screw capped vials. The vials were then shaken at 25ºC for 24 hr in a water bath. After equilibrium had been attained, the saturated solutions obtained were centrifuged to remove the excess drug (15 min, 1000 rpm). The supernatant was filtered immediately and rapidly and diluted suitably with same solvent to prevent crystallization. The filtered and diluted solutions were then analyzed spectrophotometrically at 279 nm.

6. *In-vitro* Drug Diffusion Study Through Egg Membrane[55]

Preparation of egg membrane: From local department store egg was purchased. The egg yolk was separated carefully by means of hole on the surface of the egg. After that the egg shell was immersed in 0.1N HCl for 2 hours with constant stirring followed by the complete separation of egg membrane. The membrane was washed with phosphate buffer of pH 7.4 and further used for the experimental work.

Drug diffusion through egg membrane: The *in-vitro* diffusion study was done by using Franz Diffusion Cell. The egg membrane was mounted between the donor and receptor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37˚C and was constantly stirred by using a magnetic stirrer. 1g of phytosome were placed on the egg membrane. At each sampling interval, samples were withdrawn for a period of 1 hours and

were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analyzed spectrophotometrically at 279nm

7. *Ex-vivo* Skin Permeation Study[55](optimized batch)

The permeation of *Morinda citrifolia* extract from phytosomal complex was investigated by using *in-vitro* Franz Diffusion Cell . The abdominal chicken kin was obtained from slaughter house and adhering subcutaneous fat was carefully cleaned. To remove the extraneous debris and leachable enzymes, the dermal side of the skin was kept in contact with physiological saline solution for 1 hour before starting the permeation experiment.

The skin was mounted on the receptor compartment with the stratum corneum facing towards the donor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37˚C and was constantly stirred a magnetic stirrer. 1g of phytosome were placed on the skin in the donor compartment. At each sampling interval, samples were withdrawn for a period of 10 hours and were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analyzed spectrophotometrically at 279nm.

8. Scanning Electron Microcopy (SEM) Analysis[46]

To detect the surface morphology of the phytosome, SEM of complex was performed by Scanning Electron Microscope JSM 6390 (JEOL, Japan) at STIC, Cochin University, Ernakulam . The powder samples of phytosomes was sprinkled onto the tape. The aluminum stubs were placed in the vacuum chamber of scanning electron microscope. The sample was observed for morphological characterization using secondary electron detector attached to scanning electron microscopy.

FORMULATION OF GELS OF PHYTOSOME COMPLEX[43]

- **Preparation of gel:** Gel bases were prepared by separately dispersing Carbopol 934 in distilled water with constant stirring at a moderate speed using mechanical shaker. The pH of all the formulations was adjusted to 5.5 - 6.5 using triethanolamine
- **Incorporation of Phytosomal complex into the gel:** The solution of phytosome complex was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol base. Different formulations were prepared using varying concentration of

gelling agent. Prepared gels were stored in suitable containers at room temperature for further studies.

Table No:6 Formulation of Gels of Phytosome Complex

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Carbopol 934	1%	1.5%	2%	2.5%	3%	3.5%
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Propyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Ethanol	1%	1%	1%	1%	1%	1%
Distilled Water	q.s	q.s	q.s	q.s	q.s	q.s

EVALUATION OF GELS OF PHYTOSOME COMPLEX

1. Homogeneity[55]

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container .They were tested for their appearance and presence of any aggregates.

2. Measurement of pH[41]

The pH of the phytosome gels were measured with the help of digital pH meter. 0.5 g of phytosome gel was dissolved in 50 ml of distilled water and stored for two hrs. The measurement of pH of each formulation was determined.

3. Drug content[43]

1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 279 nm.

4. Rheological study[41]

The measurements of viscosity of prepared gels were carried out with Brookfield Viscometer (spindle type S–96). The readings of each formulation were taken.

5. Spreadability[43]

On a glass plate of 10×5cm, 350mg emulgel was taken and another plate of same sized was dropped from a distance of 5cm. After 1 minute the diameter of the circle spread was measured.

6. Extrudability[43]

In the present study, extrudability was determined by measuring the weight (in grams) required to extrude at least 0.5cm gel from lacquered aluminum collapsible tube in 10 sec . The extrudability was then calculated by using the following equation:

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (in gram)}}{\text{Area(in cm}^2\text{)}} \quad \textbf{.....(3)}$$

7. *In-vitro* drug release study[55]

The *in-vitro* rug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on egg membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer pH 5.5 was used as diffusion media. The temperature of the cell was maintained at 37˚C. The whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. One ml of aliquots were withdrawn from the diffusion medium at specific time interval for 12 hours and same quantity of fresh , pre-warmed diffusion medium was replaced for the amount withdrawn. The samples withdrawn were analyzed spectrophotometrically at 279 nm and the cumulative % drug release was calculated.

8. Drug Release Kinetics

To know the release kinetics, the data obtained from the in-vitro release profile was fitted into various models like :

- Zero order kinetic model: cumulative percent drug release v/s time
- First order kinetic model: log cumulative percent drug remaining v/s time
- Higuchi's model: cumulative percent drug release v/s square root of time
- Korsmeyer - Peppas model: log cumulative percent drug release v/s log time

Zero order kinetics:

It describes the system in which the drug release rate is independent of its concentration.

$$\mathbf{Q_t = Q_0 + K_0 t} \qquad \textbf{.....(4)}$$

Where,

Q_t = Amount of drug dissolved in time t

Q_0 = Initial amount of drug in the solution, which is often 0

K_0 =Zero order release constant

If the release pattern obeys zero order, then the plot of Q_t v/s t will give a straight line with a slope of K_0 and an intercept at 0.

First order kinetics

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\mathbf{\log Q_t = \log Q_0 + kt/2.303} \qquad \textbf{.....(5)}$$

Where,

Q_t = Amount of drug released in time t

Q_0 = Initial amount of drug in the solution

K = First order release constant

If the release pattern obeys first order, then the plot of log (Q_0-Q_t) v/s t will be straight line with a slope of kt/2.303 and an intercept at t = log Q_0.

Higuchi model

According to this model, the fraction of drug from the system is proportional to the square root of time.

$$M_t/M_\alpha = kHt^{1/2} \quad \text{.....(6)}$$

Where, M_t & Mα = Cumulative amounts of drug release at time t and at infinity

kH = Higuchi dissolution constant (reflects formulation characteristics)

If the Higuchi model of drug release is obeyed, then a plot of Mt/M ∞ v/s t ½ will be straight line with slope of kH.

Korsmeyer – Peppas model (power law)

The power law describes the drug release from the polymeric system in which the release deviates from Fickian diffusion. It is expressed using the following equations :

$$M_t/M_\alpha = k_t n \quad \text{.....(7)}$$

$$\log [M_t/M_\alpha] = \log k + n \log t \quad \text{.....(8)}$$

Where, M_t & Mα = Cumulative amounts of drug release at time t and at infinity

k = Constant incorporating structural and geometrical characteristics of the system

n = Exponent determining the mechanism of drug release

To characterize the release mechanism, the dissolution data ($M_t/M_\alpha < 0.6$) are evaluated.

A plot of M_t/M_α v/s log t will be linear with slope n and intercept value of log k. Antilog of k gives the value of k. Peppas used the n value in order to characterize different release mechanisms as shown below:

Table 7: Release Mechanisms

'n' value	**Drug Release**
<0.5	Fickian
0.5<n<1	Non – Fickian
>1	Case 2 transport

STABILITY STUDIES

Stability of a drug in a dosage form at different environmental conditions is important, because it determines the expiry date of that formulation. Hence, the stability of the prepared formulation was studied. Stability studies were conducted according by storing the gel formulation at $40^{o}C \pm 2^{0}C$, 70% RH ±5% for 45 days. The samples were withdrawn at initial, 30^{th} & 45^{th} day and analyzed suitably for the physical characteristics, drug content and cumulative drug release.

RESULTS AND DISCUSSION

Calibration Curve Of *Morinda citrifolia* Extract

The λ_{max} of *Morinda citrifolia* Extract was determined by scanning the prepared solution in the wavelength range of 200-400 nm. The maximum wavelength was found to be 279nm. The calibration curve of *Morinda citrifolia* extract was constructed by dissolving the drug in pH 7.4 phosphate buffer. The linearity of the curve was found in the concentration range of 2-10µg/ml. A regression coefficient (R^2) value of 0.9989 was obtained.

Table 8: Calibration curve data of *Morinda citrifolia* extract

Concentration(µg/ml)	Absorbance
2	0.172
4	0.314
6	0.491
8	0.657
10	0.793

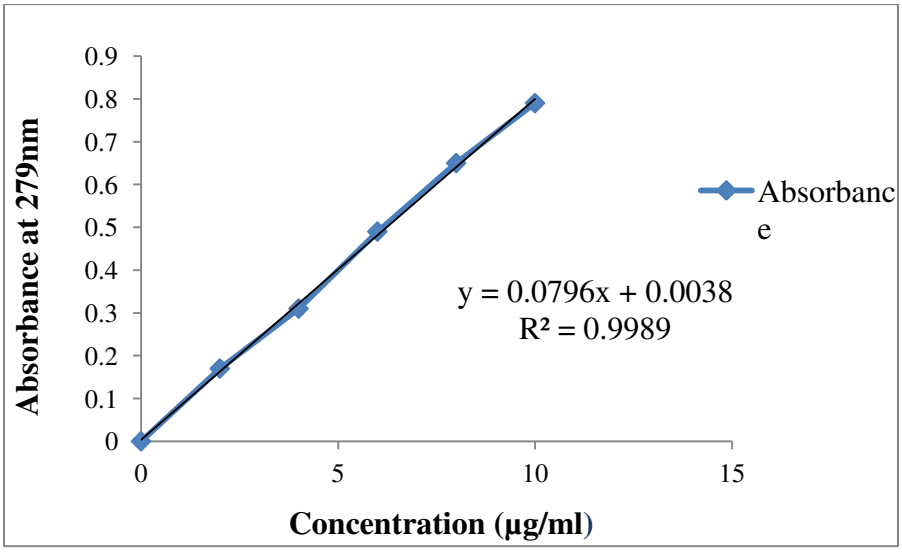


Figure No:8 Calibration curve of *Morinda citrifolia* extract

EVALUATION OF PHYTOSOMAL COMPLEX

1. Optical Microscopy

Optical microscopy was performed by viewing the formulations under microscope. It was observed that the preparations showed vesicle formation. The vesicles formed were found to be of uniform size and shape.

2. Percentage Practical Yield

Table No:9 Results of Percentage Practical Yield

Formulation	Percentage PracticalYield
F1	91.34
F2	88.51
F3	86.87
F4	86.04
F5	85.42
F6	83.87
F7	81.09

% Practical Yield of different formulations was shown in table No:7. F1 have higher % Practical yield of 91.34% than other formulations.

3. Entrapment Efficiency

Table No:10 Results of Entrapment Efficiency

Formulation	PercentageEntrapment Efficiency
F1	89.87
F2	86.94
F3	84.71
F4	79.09
F5	75.08
F6	71.58
F7	67.47

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulation F1 showed highest release entrapment efficiency of 89.87% indicating the optimum amount of lipid required for the formation of phytosomes. With further increase in the lipid concentration, the entrapment efficiency decreased indicating that the lipid concentration did not help in entrapping the drug into the matrix.

4. Drug Content

Table No: 11 Results of Drug Content

Formulation	Drug Content (%W/W)
F1	88.43
F2	86.84
F3	86.22
F4	84.19
F5	83.49
F6	80.76
F7	78.08

The drug content of *Morinda citrifolia* extract in the complexes was found to be in the range of 88.43% - 78.08% indicating the presence of an acceptable amount of drug in the formulations. The percentage of drug loading decreased with an increase in the concentration of lipid. The formulation F_1 showed the maximum drug content of 88.43%.

5. Solubility Determination

The solubility of the *Morinda citrifolia* phytosomes was found to be much higher than the pure drug extract. The increase in solubility of drug extract in the complex can be explained by the solubilization theory resulted from the formation of micelle in the medium and also by the amorphous nature of the complex. These amphiphilic surfactants (phospholipids) may increase the solubility of the drug extract by their wetting and dispersion properties. The formulation F_1 exhibited the highest degree of solubility.

Table No:12 Solubility profile in different media

Formulation	Solubility in Water (mg/ml)	Solubility in pH7.4 Phosphate Buffer (mg/ml)	Solubility in n-Octanol (mg/ml)
Drug Extract	0.143	0.197	0.231
F1	0.789	5.273	5.976
F2	0.781	5.151	5.640
F3	0.652	4.837	5.284
F4	0.694	3.950	4.569
F5	0.528	3.752	4.191
F6	0.573	3.864	4.237
F7	0.617	4.356	4.587

6. *In-vitro* Drug Diffusion Study of Phytosomes

Table No:13 Results of *In-vitro* Drug Diffusion Study

Time in hrs	Pure drug extract	F1	F2	F3	F4	F5	F6	F7
0.25	2.51	4.6	3.61	3.22	2.85	3.26	3.95	3.07
0.5	8.66	11.58	10.37	9.88	8.64	9.54	7.85	7.33
1	12.33	21.29	20.07	18.96	15.37	16.73	14.26	13.42
2	15.06	33.16	27.68	25.65	23.03	23.46	20.11	16.66
3	20.49	40.64	38.42	36.19	29.31	31.51	26.33	25.13
4	27.86	51.36	44.74	42.34	37.77	39.22	35.21	31.05
5	31.15	58.61	50.32	49.25	42.08	45.58	40.29	39.11
6	36.4	65.77	57.35	53.64	48.2	50.96	48.07	45.28
7	39.22	75.31	66.32	62.31	57.54	55.18	52.14	51.11
8	46.78	84.33	74.62	69.23	62.03	64.41	60.17	58.19
9	50.22	89.17	82.79	77.01	68.56	72.33	65.23	61.13
10	54.16	91.23	86.34	81.26	72.48	76.23	70.03	65.31

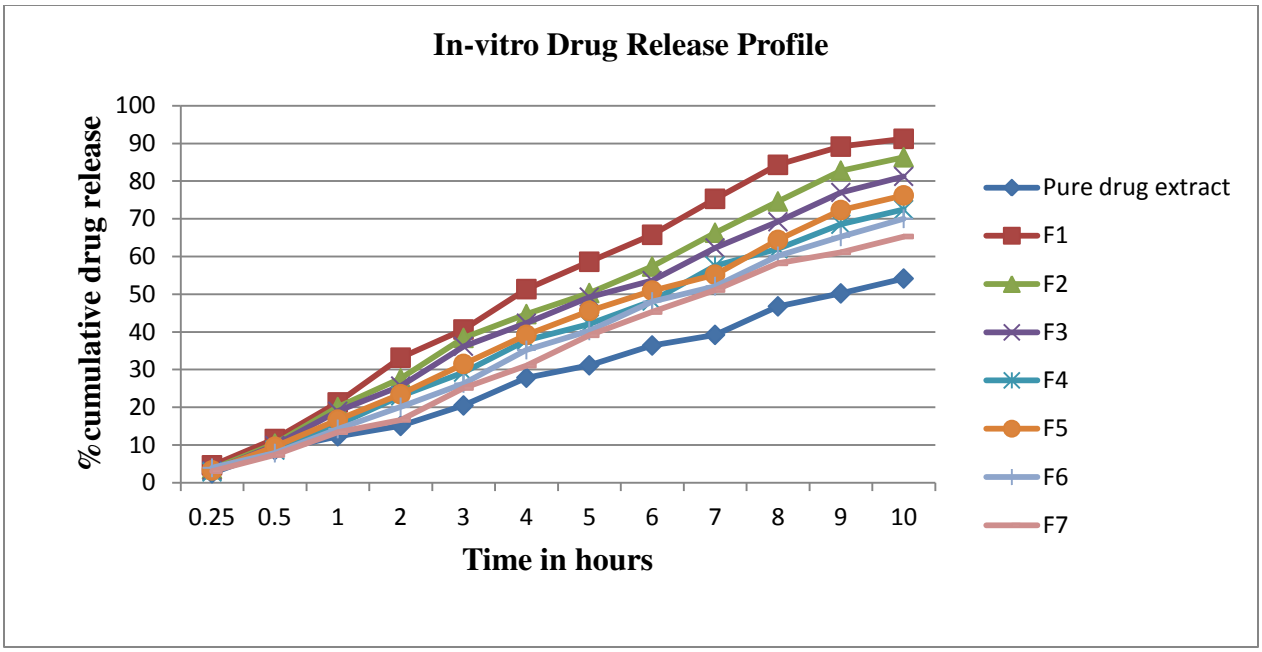


Figure No:9 *In-vitro* Drug Diffusion Profile

The phytosomes of *Morinda citrifolia* showed better diffusion profile than the pure drug extract. Unlike the free drug extract (which showed a total of only 54.16% drug release at the end of the 10 hour), all the formulations showed the percentage cumulative drug release in the range of 65.31 – 91.23%. The formulation F_1 with drug extract: soya lecithin ratio of 1:1 showed the maximum release of 91.23% at the 10^{th} hour. The diffusion of drug particles from its dosage form is a complex operation influenced by a number of factors like the particle size, crystal habit, surface area, surface energies and wettability. Wetting and dispersion properties of phospholipids (an amphiphilic surfactant) increased the solubility of the drug and hence improved the diffusion profile of the complex.

7. *Ex-vivo* Skin Permeation Study

Optimized formulation F1 was further subjected to the following studies:

Table No:14 Results of *Ex-vivo* Skin Permeation Study

Time in hours	Cumulative % permeation
0.25	5.36
0.5	13.08
1	21.76
2	27.43
3	34.22
4	40.24
5	46.03
6	51.07
7	59.35
8	66.1
9	73.46
10	83.51
11	90.23
12	92.57

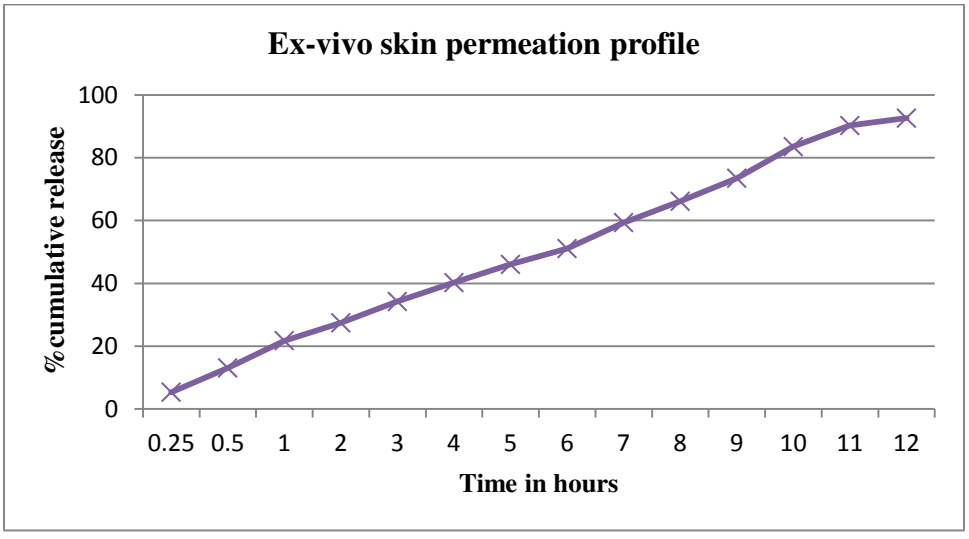


Figure No:10 Drug Permeation Profile of F1

The drug permeation of the formulation F1 through the abdominal skin of chicken was carried using Franz diffusion cell and the results are reported in table no:12. The permeation profile was plotted between% cumulative drug permeated v/s time. It was observed that the formulation showed an optimum release of 92.57% over a period of 12 hours.

8. Scanning Electron Microcopy (SEM) Analysis

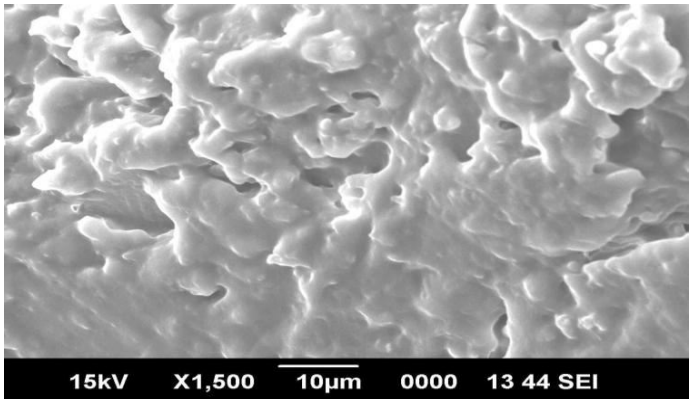


Figure No:11 SEM image of *Morinda citrifolia* Phytosome(F1)

The surface morphology of the formulated phytosome (optimized formulation)were confirmed by scanning electron microscopy. The vesicles are spherical in shape and smooth in nature.

EVALUATION OF GELS OF PHYTOSOME COMPLEX

1. Homogeneity

Table No:15 Results of Homogeneity of Different Gel Formulations

Formulation	Homogeneity
F1	Good
F2	Good
F3	Good
F4	Good
F5	Good
F6	Good

The visual inspection of all the prepared gel formulations were carried out and it was concluded that all the gel formulations showed good appearance and homogeneity.

2. Measurement of pH

Table No:16 Results of pH of different gel formulations

Formulation	pH
F1	5.4
F2	5.2
F3	5.3
F4	5.7
F5	5.1
F6	5.6

The pH of the gel formulations was in the range of 5.4 to 5.7, which lies in the normal pH range of the skin and would not produce any skin irritation.

3. Drug Content

Table No:17 Results of Drug Content of different Gel Formulation

Formulation	Drug Content(%)
F1	90.29
F2	89.17
F3	87.79
F4	87.42
F5	85.21
F6	82.37

The drug content of the formulated gels was estimated spectrophotometrically at 279nm. The drug content of all the formulation was found to be in the range of 82.37 % to 90.29% in which the best formulation F1 contained 90.29% of the drug.

4. Rheological study

Table No:18 Results of Viscosity of Different Gel Formulations

Formulation	Viscosity(Centipoise)
F1	9564
F2	10672
F3	11296
F4	12420
F5	12717
F6	13619

The gel was rotated at 50 rpm for 10 minutes with spindle 64. The corresponding reading was noted. The viscosity of the formulations increases as concentration of polymer increases. The viscosity of the best formulation was found to be 9564 centipoise.

5. Spreadability

Table No:19 Results of Spredability of Different Gel Formulations

Formulation	Spreadability(cm)
F1	4.1
F2	3.8
F3	3.3
F4	3.6
F5	3.5
F6	2.9

Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreadability of the prepared gel formulations was carried out and it was concluded that all the formulation showed acceptable spreadability. The spreadability coefficient of the best formulation F1 was found to be 4.1cm. The value of spreadability indicates the gel was easily spreadable by small amount of shear.

6. Extrudability

Table No:20 Results of Extrudability of Different Gel Formulations

Formulation	Extrudability(gm/cm^2)
F1	9.3
F2	11.2
F3	13.1
F4	13.7
F5	14.3
F6	14.7

It was found that extrudability of the prepared gel formulation was a function of concentration of gelling agents. Extrudability was decreased with increase in concentration of gelling agents. The extrudability of the best formulation F1 was found to be 9.3 gm/cm^2. Thus the prepared gel posses optimum extrudability.

7. *In-vitro* drug release study

The *in-vitro* permeation studies of all the formulations were carried out using Franz diffusion cell wtih egg membrane as a diffusion membrane for the study as described in the methodology section.

The comparative data of cumulative % drug permeation of the formulations were shown in table no: 20 and the figure no: 11. Cumulative % drug release of the prepared gel formulations after12 hours was found to be in the range of 66.32% to 89.95%. The drug release from F1 was found to be higher due to the lower concentration of the gelling agent and F6 showed lower drug release due to the higher concentration of the gelling agent.

Table No: 21 Results of *In-vitro* drug release study of Gel Formulations

Time in hours	F1	F2	F3	F4	F5	F6
0.5	10.36	9.11	8.47	7.23	5.92	5.92
1.5	12.63	11.06	10.34	9.24	8.03	7.34
1	18.07	15.07	13.36	11.65	10.56	9.18
2	24.1	20.15	17.54	14.87	13.76	10.94
3	30.17	24.65	20.54	18.65	17.45	14.23
4	35.17	27.67	22.45	20.87	20.12	17.65
5	38.14	30.65	27.34	26.56	25.43	23.01
6	43.58	35.65	33.44	30.67	29.54	26.25
7	52.07	39.78	36.55	35.42	34.76	31.11
8	59.34	48.19	44.13	40.63	38.95	35.78
9	67.46	58.74	52.85	48.63	45.65	40.67
10	74.02	66.13	59.65	57.57	53.23	49.63
11	84.27	74.16	68.13	65.13	61.76	60.54
12	89.35	80.56	73.48	69.03	68.49	66.32

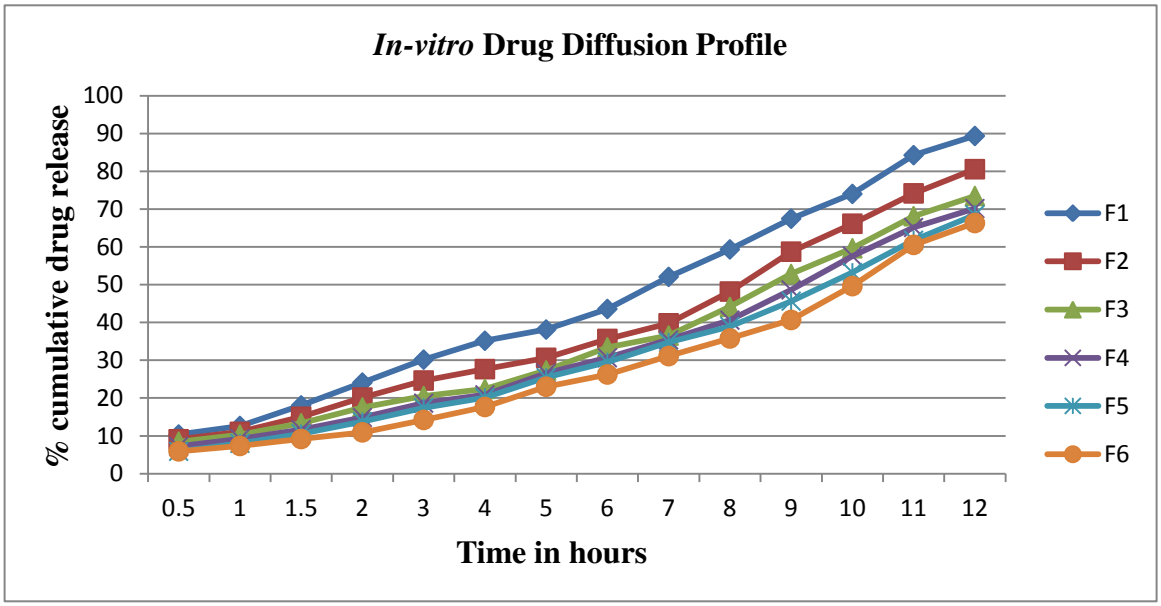


Figure No:12 *In-vitro* Drug Diffusion Profile of Gel Formulation

8. Drug Release Kinetics

Based on the data obtained from the *in-vitro* drug release studies the best formulation F_1 was analyzed for the release kinetic studies. The cumulative release of drug was fitted into various plots like Zero order, First order and Higuchi model to know the pattern of release and Korsmeyer-Peppas model in order to find out the mechanism of release from the prepared pharmacosome. The model that best fits the release data is selected based on the regression coefficient value of various models.

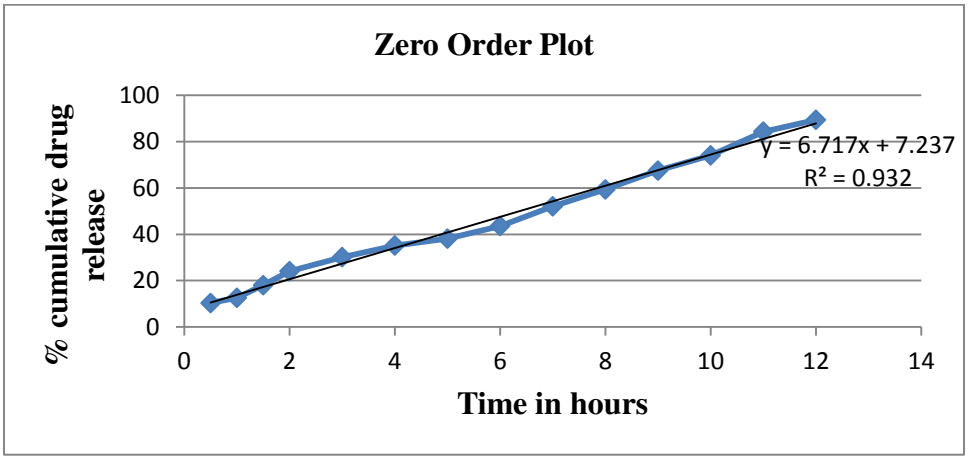


Figure No:13 Zero Order Plot

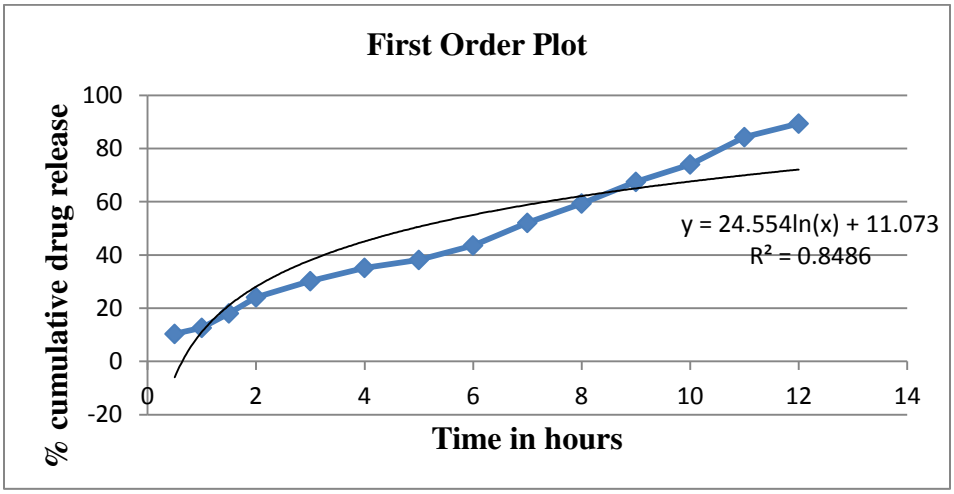


Figure No:14 First Order Plot

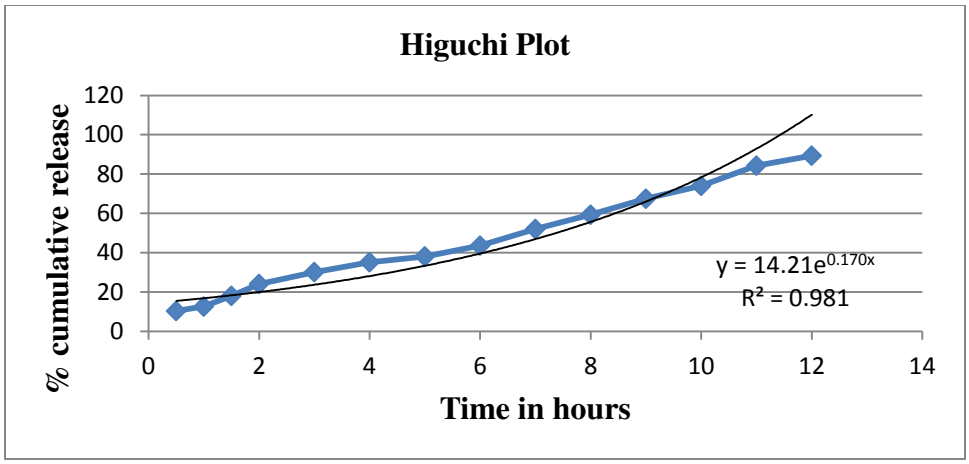


Figure No:15 Higuchi Plot

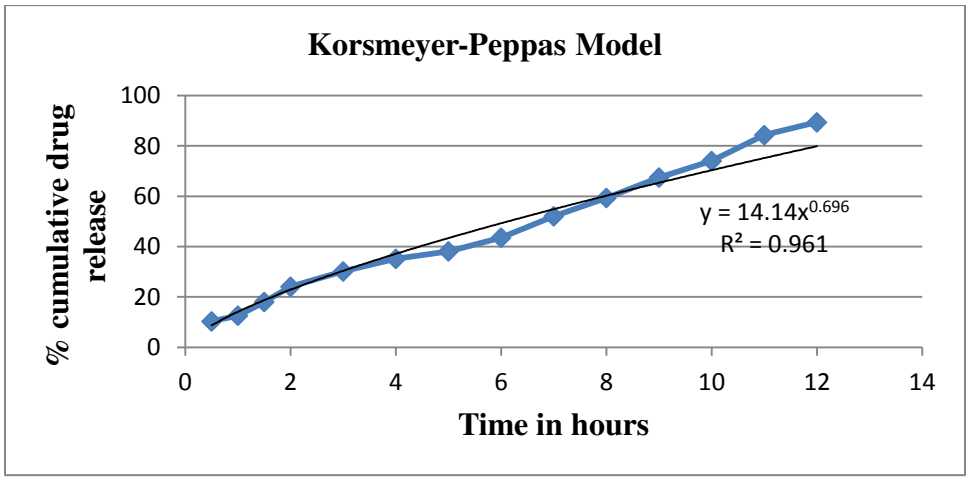


Figure No: 16 Korsmeyer-Peppas Plot

Table No:22 Results of kinetic analysis

Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	
	R^2	R^2	R^2	n	R^2
F_1	0.932	0.848	0.981	0.696	0.961

From the regression coefficient values obtained, it was found out that the formulation follows the Higuchi model kinetics. The slope value (n) obtained from Peppas plot was 0.696, which indicates that the formulation followed Non-Fickian diffusion mechanism of drug release.

9. Stability Studies

Table No:23 Stability Studies For F1 Formulation

Sl.No	Parameters	Initial	30th Day	45th Day
1	Homogeneity	Good	Good	Good
2	Drug Content(%)	90.29	90.23	90.23
3	pH	5.4	5.4	5.4
4	Spreadability(cm)	4.1	4.1	4
5	Extrudability(gm/cm^2)	9.3	9.3	9.3
6	Viscosity (cps)	9564	9561	9555
7	% cumulative release	89.35	89.30	88.83

The purpose of the stability testing is to provide evidence on how the quality of a drug substance or drug varies with time under the influence of variety of environmental factors like temperature, humidity and light and to establish a test period for the drug substance or a shelf life for the drug and recommended storage conditions. Here the gel are packed in collapsible aluminum tubes and were loaded at accelerated condition at 40 ± 2°C/ RH 70 ± 5% RH in a stability chamber. Samples were withdrawn at initial 40 ± 2°C/ RH 70 ± 5% and days and evaluated for homogeneity, drug content, pH, spreadability, extrudability, viscosity and in-vitro diffusion profile. The results showed that the storage at these conditions had no effect on those parameters.

SUMMARY

Morinda citrifolia L. (Noni) have been used for thousands of years for the treatment of many health problems including cancer, cold, diabetes, flu, hypertension and pain. Scientific investigations reported its antioxidant, antifungal , anti bacterial, anti-inflammatory, liver protective, anticancer, analgesic, immunomodulatory, anti viral and wound healing activities. Even though extracts have reported several therapeutic benefits, but extraction of individual compound from it often exhibits limited clinical utility as the synergistic effect of various natural ingredients gets lost and the various phytoconstituents present in it are poorly absorbed either due to their large molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability.

Phytosome technology has proved to be beneficial in providing better absorption and bioavailability of polar biomolecules over conventional herbal extracts. Hence the present study was aimed to prepare and evaluate topical phytosomal gel of *Morinda citrifolia* with an objective to increase its bioavailability and therapeutic efficacy.

Formulation

Morinda citrifolia phytosomes was prepared by the anti solvent precipitation technique using different ratios of drug and soya lecithin. A total of 7 formulations were prepared. From the prepared formulation the best formulation which contained drug extract: soya lecithin in the ratio 1:1 was selected based on various evaluation parameters and was incorporated into a gel base of different concentration using carbopol 934 as a polymer.

UV spectrophotometric method was developed for determining λ_{max} *of Morinda citrifolia* extract

Evaluation

- The phytosomal formulations were subjected to various studies like percentage yield , solubility, drug content , entrapment efficiency, *in vitro* drug release studies.

- The optimized formulation was further subjected to *ex-vivo* skin permeation study and SEM analysis.
- The prepared gel formulations was evaluated for pH, spreadability coefficient, extrudability, drug content, rheological studies and *in-vitro* drug diffusion study.
- Release kinetic data revealed that the gel followed Higuchi model kinetics with non-Fickian diffusion of drug.
- Stability studies were carried out at accelerated temperature $40^{o}C \pm 2^{0}C$, 70% RH ±5% for 45 days. There were no significant changes in the homogeneity, drug content, pH, spreadability, extrudability, viscosity and in-vitro diffusion profile.

CONCLUSION

The novel drug delivery system research area of herbal drugs is an innovative work that target for phytoconstituents and plant extracts regarding the therapeutic and cosmetic usefulness of plant products particularly containing flavonoids and poly phenolic compounds. However,due to its poor lipid solubility and larger molecular size limiting their ability to pass across the lipid-rich biological membranes, resulting poor bioavailability. Different reports show a promising future of phytosome as an advanced form of herbal products that are better absorbed, utilized, and as a result produce better results than conventional herbal extracts. The complexation of phytoconstituents and phospholipids makes the phytoconstituents more stable in the complex form due to liopophilic nature and offering the herbal drugs with sufficient lipid penetrability, higher concentration, sustained therapeutic levels and increased cosmetic value. It was confirmed that *Morinda citrifolia* phytosoml gel showed a better diffusion as well as stability profile, hence providing an attractive carrier for the delivery of various phytoconstituents present in it. In conclusion, the application of phytosomal formulation as topical pharmaceutical agent and cosmetics with improved safety and efficacy results in proper utilization of herbal drugs and cost effective pharmaceutical product.

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ABSTRACT

The emerging technology of drug delivery is being applied to phyto-pharmaceuticals to improve the bioavailability of herbal extracts for medicinal applications. Phytosome is a novel emerging technique applied to phyto-pharmaceutical which contains phytoconstituents of herbal extract surrounds and bound by lipid. Several plant extracts and phyto constituents are having excellent bioactivity *in vitro*, demonstrate less or no *in-vivo* actions due to their poor lipid solubility or larger molecular size or both, resulting poor absorption and bioavailability. So, much work has been directed towards the development of new concept in herbal delivery system i.e., phytosomes which are better absorbed, utilized and as a result produce better results than conventional herbal extracts.The design of the present investigation was to prepare and develop phytosomal complex of *Morinda citrifolia* with different ratio of drug extract and soyalecithin. A total of 7 formulations were prepared by anti solvent precipitation method and evaluated for percentage yield, solubility, drug content, entrapment efficiency, *in vitro* drug release studies and *ex-vivo* drug permeation studies. The optimized formulation of phytosome was incorporated into a gel base of different concentration and was subjected to various evaluation studies. The *in-vitro* diffusion and kinetic study of the gel showed a release of 89.35% over 12 hours and fitted into Higuchi Model Kinetics and non-Fickian diffusion mechanism. The formulation was found to be stable at an accelerated temperature of 40° ± 2°C, RH 70 %± 5 % for 45 days. It could be concluded that the formulation F1 having drug extract: soyalecithin 1:1 was the best formulation. The SEM image of the optimized formulation was taken.

Keywords: Phytosome, Phytoconstituents, Bioavailability *In vitro* drug release, *Ex-vivo* drug permeation study, SEM